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(57) Abstract

This invention relates to polypeptides displaying the activity of anti-Mullerian hormone (AMH) receptor, also known as Mullerian inhibiting substance (MIS) receptors, and antibodies to those polypeptides. More particularly, this invention relates to such AMH receptor polypeptides and antibodies, processes for producing those polypeptides and antibodies and methods for using them in the treatment of cancer and tumors of tissues associated with expression of the anti-Mullerian hormone receptor.

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ANTI-MULLERIAN HORMONE RECEPTOR POLYPEPTIDES AND ANTIBODIES THERETO

TECHNICAL FIELD OF INVENTION

This invention relates to polypeptides displaying the activity of anti-Mullerian hormone receptors and antibodies and antibody homologs to those polypeptides. More particularly, this invention relates to such polypeptides and antibodies, processes for producing those polypeptides and antibodies and methods for using them in the treatment of cancer and tumors of tissues associated with expression of the anti-Mullerian hormone receptor.

BACKGROUND OF INVENTION

Anti-Mullerian hormone (AMH), also called Mullerian inhibiting substance (MIS), is a glycoprotein produced by prepubertal Sertoli cells and by postnatal granulosa cells. It is a non-steroidal factor that causes regression of the Mullerian duct, the anlage of the internal female reproductive tract, in the male fetus. AMH is secreted at low levels by postnatal gonadal cells. The significance of postnatal AMH is not fully understood.

AMH has been hypothesized to be useful in treating tumors that derive from the Mullerian duct (e.g., uterus, Fallopian tubes) and tumors of tissues that derive from progenitor cells of the Mullerian duct (e.g., ovaries), by binding to the AMH receptor and

inhibiting cell proliferation. However, there have been problems identifying the functional AMH ligand. An alternate approach to the treatment of such tumors is to use the AMH receptor to target the tumors.

AMH is part of the transforming growth factor-ß (TGF-ß) superfamily. It is structurally and functionally related to TGF-ß, bone morphogenic protein (BMP) and activin. Receptors have been isolated for some of these ligands, including the activin type II receptor (ActR-II) (L.Mathews and W.Vale, Cell, 65, p. 973 (1993)) and the TGF-ß type II receptor (TGFßR-II) (H.Lin et al. Cell, 68, p. 775 (1992)). More recently, investigators have identified a type I receptor which may be associated with ActR-II (L.Attisano et al., Cell, 75, pp. 671-680 (1993)) and/or with TGFßR-II (R.Ebner et al., Science, 260, pp. 1344-1348 (1993)). This same receptor has also been identified as an AMH (or Mullerian inhibiting substance) receptor by W.He et al., Developmental Dynamics, 196, pp. 133-142 (1993). However, its expression profile is not at all consistent with the expected expression profile of an AMH receptor.

AMH receptors are present in a very limited number of body tissues. Therefore, the AMH receptor is particularly useful for developing antibody-toxin conjugates to target tumor treatments. Antibody-toxin complexes targeted to the AMH receptor can be used much more aggressively than antibody-toxin complexes targeted to other receptors which are typically more prevalent in the body.

SUMMARY OF INVENTION

This invention provides isolated DNA sequences encoding AMH receptor polypeptides. This invention further provides recombinant DNA molecules comprising an AMH receptor DNA sequence and capable of

directing expression of an AMH receptor polypeptide. This invention also provides isolated AMH receptor polypeptides which possesses the biological or antigenic activity of native AMH receptor.

Another aspect of this invention provides a method for detecting the AMH receptor polypeptide in vitro or in vivo comprising the steps of contacting an AMH receptor antibody with a sample or cell suspected of containing the receptor polypeptide and detecting if binding has occurred.

This invention also provides an assay for detecting the presence of the AMH ligand in a test sample by contacting the test sample with the AMH receptor polypeptide and determining whether binding has occurred.

Another aspect of this invention provides a method for identifying and then isolating and purifying molecules that bind to an AMH receptor polypeptide comprising contacting a sample containing the test molecules with an AMH receptor polypeptide immobilized on a support under conditions whereby the molecules to be identified are selectively adsorbed onto the immobilized receptor, washing the immobilized support to remove non-adsorbed material and separating the bound molecule from the immobilized AMH receptor polypeptide to which they are adsorbed.

This invention also provides antibodies and antibody homologs capable of binding to the AMH receptor polypeptide. Another aspect of this invention provides a pharmaceutical composition comprising an antibody capable of binding to the AMH receptor polypeptide conjugated with a toxin or radionuclide capable of killing or preventing growth of a cell expressing the AMH receptor polypeptide. A further aspect of this invention is a method of treating cancers or tumors in tissues characterized by the

expression of the AMH receptor polypeptide by administering an AMH receptor antibody-toxin or antibody-radionuclide conjugate.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A is a schematic diagram showing proteins encoded by three clones (7F2, 2B10 and 3D6) isolated from a fetal ovary library and by clone H1, constructed by joining the extracellular domain of 3D6 to the transmembrane kinase region of 2B10 using a BspM1 restriction site. The domain encoded by an extra exon in 3D6 is hatched. The signal peptide is shown in black and introns A (159 bp) and B (120 bp) are represented by arrowheads.

Figures 1B and 1C represent the nucleotide sequence and translated amino acid sequence of clone H1. The BspM1 restriction site used for the construction is indicated. The nucleotide sequence of the extra exon found in 3D6 is shown in lower case and its translated protein sequence is underlined. The transmembrane region is shaded and the predicted signal sequence cleavage sites for both the H1 and the 2B10 proteins are indicated by arrows. Introns A and B are indicated by arrowheads. Potential N-linked glycosylation sites are boxed. Two sites at which nucleotide differences were observed between 7F2, 2B10 and 3D6 are also indicated. H1 contains the consensus sequence at these two positions.

Figure 1D depicts a comparison of the rabbit AMH receptor encoded by H1 with the human TGF-ß type II receptor and the mouse activin type II receptor. Amino acids shared by at least two receptors are shaded. Cysteines conserved in all three extracellular domains are indicated by a dot. Roman numbers indicate protein kinase catalytic consensus domains. Domains VIB and VIII are specific for serine-threonine kinases.

Figures 2A and 2B depict Northern blot hybridization of 7F2 to mRNA extracted from various organs of developing rabbits. "SC" represents Sertoli cells.

Figures 2C-2E depict in situ hybridization of rabbit reproductive tissues: (C) adult (16 weeks) ovary containing follicles at various developmental stages; (D) 18-day-old fetal testis; and (E) 18-day-old female fetal, sexually undifferentiated reproductive tract containing Mullerian (M) and Wolffian (W) ducts.

Figure 3 depicts (A) cells transfected with H1 and exposed to plasmin-cleaved AMH; (B) cells transfected with H1 and exposed to full-length AMH; (C) cells transfected with 2B10 and exposed to plasmin-cleaved AMH; and (D) cells transfected with ß-galactosidase DNA and exposed to plasmin-cleaved AMH. Negative COS cells are indicated by arrows. Dark field illumination, x 500.

Figure 4A is a schematic diagram showing primers used for reverse-transcriptase polymerase chain (RT-PCR) reaction and the expected PCR fragments generated for the two receptor isoforms, i.e., 164 bp for 2B10 and 347 bp for H1. The PCR oligonucleotides are indicated by arrows, the sense oligonucleotide is

5' GCAGGATGCT GGGCACTCTG 3' [SEQ ID NO: 8]

and the antisense oligonucleotide is

5' GTCAGCACCA CAGGAGCAGG 3' [SEQ ID NO: 9]

Figure 4B depicts the gel analysis of RT-PCR products generated from RNAs extracted from various rabbit organs.

Figure 5 depicts a comparison of the rabbit (bottom line) [SEQ ID NO: 4] and human (top line) [SEQ ID NO: 13] AMH receptor protein sequences.

Figure 6 represents a partial nucleotide sequence of the AMH receptor gene of patient T.A. [nucleotides 401 to 800 of SEQ ID NO: 14]. The exon is

shown in upper case letters and the intron is shown in lower case letters. A G>A mutation is indicated by an arrow.

Figure 7 represents a partial nucleotide sequence [SEQ ID NO: 14] of the AMH receptor gene isolated from the λEMBL4 library. Exons are in upper case; introns are in lower case. The nucleotide that is mutated in patient T.A. is indicated by an arrow. The protein sequence is shown below the exon sequences.

Figure 8 depicts an electrophoretic analysis of RT-PCR products generated with RNA isolated from control tissues from normal individuals and with RNA from the testis of patient T.A.. The expected band is observed in the control samples while two aberrant bands are seen in the T.A. sample. The smaller band represents an mRNA that has undergone exon skipping, while the other band, which is slightly larger than the normal PCR product in the control lanes, reflects an mRNA that has undergone cryptic splicing.

Figure 9 is a schematic diagram showing the generation of the two aberrant mRNAs in patient T.A., caused by the splicing mutation in the AMH receptor gene.

Figure 10 depicts an electrophoretic analysis of RT-PCR product generated with RNA from the four human granulosa cell tumors. Lane 1 -- ovarian tissue, patient 1; Lane 2 -- ovarian tissue, patient 2; Lane 3 -- ovarian tissue, patient 3; Lane 4 -- metastasis, patient 3; Lane 5 -- size marker PhiX-Hae III. The expected band is seen clearly in lanes 1 and 2 and more faintly in lanes 3 and 4, indicating that the tumors express the AMH receptor.

DETAILED DESCRIPTION OF THE INVENTION

An isolated AMH receptor DNA sequence is a DNA sequence that is identified and separated from at

least one contaminant DNA sequence with which it is ordinarily associated in the natural source of the AMH receptor. An isolated AMH receptor DNA sequence is other than in the form or setting in which it is found in nature. An isolated AMH receptor DNA sequence therefore is distinguished from the AMH receptor DNA sequence as it exists in natural cells.

Preferably, the isolated AMH receptor DNA sequence comprises a DNA sequence encoding the mature AMH receptor polypeptide selected from the group consisting of

(a) nucleotides 113 to 1585 of SEQ ID NO: 1;

(b) nucleotides 110 to 1765 of SEQ ID NO: 2;

(c) nucleotides 112 to 1779 of SEQ ID NO: 12;

(d) DNA sequences that hybridize to any of the DNA sequences of (a)-(c) and which encode a biologically or antigenically active AMH receptor polypeptide; and

(e) DNA sequences that are degenerate to any of the foregoing DNA sequences.

Alternatively, the isolated AMH receptor DNA sequence comprises a DNA sequence encoding the extracellular domain of the AMH receptor polypeptide selected from the group consisting of

(a) nucleotides 113-310 of SEQ ID NO: 1;

(b) nucleotides 110-490 of SEQ ID NO: 2;

(c) nucleotides 112-492 of SEQ ID NO: 12;

(d) DNA sequences that hybridize to any of the DNA sequences of (a)-(c) and which encode a biologically or antigenically active AMH receptor polypeptide; and

(e) DNA sequences that are degenerate to any of the foregoing DNA sequences.

"Hybridization" as used in this application means hybridization carried out under conditions of high or moderate stringency. High stringency

conditions are defined as hybridizing with plaque screen buffer (0.2% polyvinylpyrrolidone, 0.2% Ficoll-400, 0.2% bovine serum albumin, 50mM Tris-HCl (pH 7.5), 1M NaCl, 0.1% sodium pyrophosphate, 1% SDS), 10% dextran sulphate, and 100 μg/ml denatured, sonicated salmon sperm DNA at 65°C for 12-20 hours, and washing with 75 mM NaCl/7.5 mM sodium citrate (0.5 X SCC)/1% SDS at 65°C. Moderate stringency conditions are defined as hybridizing with plaque screen buffer, 10% dextran sulphate and 100 μg/ml denatured, sonicated salmon sperm DNA at 55°C for 12-20 hours, and washing with 300 mM NaCl/30 mM sodium citrate (2.0 X SCC)/1% SDS at 55°C.

The isolated AMH receptor polypeptides of this invention are polypeptides that possess the biological or antigenic activity of native AMH receptor. The biological activity of the native AMH receptor is the ability to bind to AMH ligand. The antigenic activity of the native AMH receptor is the ability to raise antibody that binds with the receptor. The isolated AMH receptor polypeptides of this invention are separated from at least one contaminant polypeptide with which they are ordinarily associated in the natural source of the AMH receptor. An isolated AMH receptor polypeptide is other than in the form or setting in which it is found in nature. An isolated AMH receptor polypeptide therefore is distinguished from the AMH receptor polypeptide as it exists in natural cells.

The AMH receptor polypeptides of this invention may exist in monomeric or oligomeric forms. Oligomeric forms may be composed of only the AMH polypeptide or may include other TGF-ß superfamily type I receptors.

Preferably the AMH receptor polypeptides of this invention are selected from the group consisting

of polypeptides encoded by the isolated AMH receptor DNA sequences of this invention. More preferably, an AMH receptor polypeptide of this invention comprises an amino acid sequence corresponding to the mature AMH receptor polypeptide selected from the group consisting of

(a) amino acid residues 18 to 508 of SEQ ID NO: 3;

(b) amino acid residues 18 to 569 of SEQ ID NO: 4; and

(c) amino acid residues 18 to 573 of SEQ ID NO: 13.

Alternatively, an isolated AMH receptor polypeptides of this invention comprises an amino acid sequence corresponding to the extracellular domain the mature AMH receptor polypeptide selected from the group consisting of

(a) amino acid residues 18 to 83 of SEQ ID NO: 3;

(b) amino acid residues 18 to 144 of SEQ ID NO: 4; and

(c) amino acid residues 18 to 144 of SEQ ID NO: 13.

An antibody capable of binding to AMH receptor polypeptide is an antibody that binds to the polypeptide and is identified and separated and/or recovered from a component of any natural environment in which it may be present. Preferably, such antibody is capable of binding to the extracellular domain of the AMH receptor polypeptide. More preferably, such antibody is capable of binding to the AMH receptor in its native conformation on the surface of cells.

An antibody homolog is a protein comprising one or more polypeptides selected from immunoglobulin light chains, immunoglobulin heavy chains, and antigen-binding fragments thereof, which are capable of binding to one or more antigens. The component polypeptides of

an antibody homolog composed of more than one polypeptide may optionally be disulfide-bound or otherwise covalently crosslinked. Accordingly, antibody homologs include intact immunoglobulins of types IgA, IgG, IgE, IgD, IgM (as well as subtypes thereof), wherein the light chains of the immunoglobulin may be of types kappa or lambda. Antibody homologs also include portions of intact immunoglobulins that retain antigen-binding specificity, for example, Fab fragments, Fab' fragments, $F(ab')_2$ fragments, F(v) fragments, heavy chain monomers or dimers, light chain monomers or dimers, dimers consisting of one heavy and one light chain, and the like.

Also encompassed in the term "antibody homologs" are humanized recombinant antibody homologs and chimeric recombinant antibody homologs. A humanized recombinant antibody homolog is an antibody homolog initially derived from a nonhuman mammal in which recombinant DNA technology has been used to substitute some or all of the amino acids not required for AMH receptor binding with amino acids from corresponding regions of a human immunoglobulin light or heavy chain. A chimeric recombinant antibody homolog is an antibody homolog derived initially from a nonhuman mammal, in which recombinant DNA technology has been used to replace all or part of the hinge and constant regions of the light chain, the heavy chain or both, with corresponding regions from an immunoglobulin light chain or heavy chain of a mammal of a different species, preferably human.

The DNA sequences encoding the AMH receptor may be used as diagnostic tools to determine the extent and rate of the expression of the AMH receptor in cells of a patient. To accomplish this assay, a sample of a patient's cells is treated, via in situ hybridization,

or by other suitable means, and analyzed to determine whether the sample contains mRNA molecules capable of hybridizing with the DNA sequence encoding the AMH receptor.

The DNA sequences encoding the AMH receptor may also be used to construct recombinant DNA molecules capable of expressing the AMH polypeptides of this invention in hosts transformed therewith. A DNA sequence encoding an AMH receptor polypeptide of this invention must be operatively linked to an expression control sequence within the recombinant DNA molecule to effect such expression. The term "operatively linked" as used herein refers to positioning in a vector such that transcription and translation of the coding sequence is directed by the control sequence.

To construct a recombinant DNA molecule capable of directing expression of the AMH receptor polypeptides of this invention, the DNA sequences encoding these polypeptides may be inserted into and expressed using a wide variety of vectors. Furthermore, within each specific expression vector, various sites may be selected for insertion of these DNA sequences. These sites are usually designated by the restriction endonuclease which cuts them. They are well recognized by those of skill in the art. It will be appreciated, however, that an expression vector useful in this invention need not have a restriction endonuclease site for insertion of the chosen DNA fragment. Instead, the vector may be joined to the fragment by alternative means

The expression vector, and in particular, the site chosen for insertion of a selected DNA fragment and operative linking to an expression control sequence, is determined by a variety of factors. These factors include, e.g., the number of sites susceptible to a particular restriction enzyme, the size of the

polypeptide to be expressed, susceptibility of the desired polypeptide to proteolytic degradation by host cell enzymes, contamination or binding of the polypeptide to be expressed by host cell proteins difficult to remove during purification, expression characteristics, such as the location of start and stop codons relative to the vector sequences, and other factors recognized by those skilled in the art. The choice of vector and an insertion site for a DNA sequence is determined by a balance of these factors and not all selections will be equally effective for a given case.

Useful expression vectors may consist of segments of chromosomal, non-chromosomal and synthetic DNA sequences. Suitable expression vectors for eukaryotic hosts include, for example, vectors comprising sequences from SV40, bovine papilloma virus, adenovirus and cytomegalovirus, and vectors useful specifically in insect cells, such as pVL 941. Useful bacterial expression vectors include known bacterial plasmids, e.g., plasmids from E.coli including colE1, pCR1, pBR322, pMB9 and their derivatives; wider host range plasmids, such as RP4; the numerous derivatives of phage lambda, e.g., NM989 and the lambda gt series; other DNA phages, e.g., M13 and other filamentous single-stranded DNA phages; and commercially available high expression vectors, e.g., the pGEM series and the lambda Zap vectors. Useful mammalian cell expression vectors include, for example, the 2μ plasmid and derivatives thereof.

Such expression vectors are also characterized by at least one expression control sequence that may be operatively linked to the DNA sequences of this invention inserted in the vector in order to control and to regulate the expression of that cloned DNA sequence. Examples of useful expression

control sequences include the malE system, the OmpA system, the lac system, the trp system, the tac system, the trc system, major operator and promoter regions of phage lambda, the control region of fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase, (e.g., Pho5), the promoters of the yeast mating factors, and promoters derived from polyoma, adenovirus, retrovirus, and simian virus, e.g., the early and late promoters of SV40, eukaryotic cell promoters, such as the metallothionein promoter and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells and their viruses or combinations thereof.

The recombinant DNA molecules of the present invention may also comprise other DNA coding sequences fused to and in frame with the DNA sequences of this invention. For example, such constructs may be characterized by an ATG start codon fused directly to the nucleotides encoding the first amino acid of the mature AMH receptor polypeptide. This construction may produce an f-Met polypeptide. However, it will be understood that the initial methionine may be cleaved during expression in a transformed host or may be subsequently removed. Alternatively, a DNA sequence encoding a bacterial or eukaryotic signal sequence may be fused to the 5' end of a DNA sequence encoding the mature AMH receptor polypeptide of this invention. This would allow the expressed product to be either secreted or targeted to a specific subcellular compartment within the host cell. Most signal sequences are removed by the host cell after performing their targeting function, thus obviating the need for removal after purification of the desired polypeptide. Many signal sequences, as well as the DNA sequences encoding them, are known in the art. The fusion of

such signal sequence DNA to and in frame with the sequence encoding a mature AMH receptor polypeptide of this invention can be achieved by standard molecular biology techniques. Preferably, the signal sequence is selected from the group consisting of nucleotides 62 to 112 of SEQ ID NO: 1; nucleotides 59 to 109 of SEQ ID NO: 2; and nucleotides 61 to 111 of SEQ ID NO: 12.

Alternatively, a DNA sequence encoding an AMH receptor polypeptide of this invention may be expressed as a fusion protein by in-frame ligation to a second DNA sequence encoding a host cell polypeptide. The expression of a fusion protein may afford several advantages, such as increased resistance to host cell degradation, ease of identification based upon the activity or antigenicity of the host cell polypeptide, and ease of purification, based upon the physical or immunological properties of the host cell polypeptide.

This invention also relates to hosts transformed with the recombinant DNA molecules described above. Useful hosts which may be transformed with these recombinant DNA molecules and which may be employed to express the AMH receptor polypeptides of this invention may include well known eukaryotic and prokaryotic hosts, such as strains of <u>E.coli</u>, strains of <u>Pseudomonas</u>; strains of <u>Bacillus</u>; strains of <u>Streptomyces</u>; strains of <u>Saccharomyces</u>; animal cells such as COS cells, CHO cells, BHK cells, R1.1 cells, B-W and L-M cells, African Green Monkey kidney cells (e.g., COS 1, COS 7, BSC1, BSC40 and BMT10); human tissue cells; insect cells (e.g., <u>Spodoptera</u> <u>frugiperda</u> (SF9)); and plant cells in tissue culture. The preferred host for expression of the AMH receptor polypeptides of this invention is CHO cells.

It will be appreciated that not all host/expression vector combinations will function with equal efficiency of expression DNA sequences encoding

the AMH receptor polypeptides of this invention. However, a particular selection of a host-expression vector combination may be made by those of skill in the art after due consideration of the principles set forth herein without departing from the scope of this invention. For example, the selection should be based on a balancing of a number of factors. These factors include, for example, compatibility of the host and vector, toxicity of the polypeptides encoded by the AMH receptor DNA sequences to the host, vector copy number and the ability to control that copy number, the expression of other proteins encoded by the vector, such as antibiotic markers, ease of recovery of the desired polypeptide, expression characteristics of the DNA sequences and the expression control sequences operatively linked to them, biosafety, costs and folding or any other necessary post-expression modifications of the desired polypeptide.

While recombinant DNA techniques are the preferred method of producing the AMH receptor polypeptides of this invention, the AMH receptor DNA sequences of this invention, particularly the DNA sequences encoding only the extracellular domain of the AMH receptor polypeptides , i.e., nucleotides 113 to 310 of SEQ ID NO: 1; nucleotides 110 to 490 of SEQ ID NO: 2; and nucleotides 112 to 492 of SEQ ID NO: 12; or sequences which are degenerate to those sequences may be produced by conventional chemical synthesis techniques. Synthetically produced polypeptides of this invention can advantageously be obtained in extremely high yields and be easily purified.

In a preferred embodiment of this invention, AMH receptor polypeptides corresponding only to the extracellular domain (i.e, residues 18 to 83 of SEQ ID NO: 3; residues 18 to 144 of SEQ ID NO: 4 and residues 18 to 144 of SEQ ID NO: 13) are synthesized by solution

phase or solid phase polypeptide synthesis. Proper folding of the polypeptides may be achieved under oxidative conditions which favor disulfide bridge formation, as described by S.Kent, Ann. Rev. Biochem., 57, pp. 957-989 (1988). Polypeptides produced in this way may be purified by separation techniques widely known in the art, preferably utilizing reverse phase HPLC.

In another embodiment of this invention, fusion polypeptides, and DNA sequences coding for them are provided. These fusions have an amino-terminal region characterized by the amino acid sequence of the extracellular domain of the AMH receptor polypeptides of this invention and a carboxy terminal region comprising a domain of a protein or polypeptide other than an AMH receptor polypeptide. Such domains include, for example, the Fc region of an immunoglobulin.

In a preferred embodiment of this invention, the extracellular domain of the AMH receptor polypeptides of this invention are fused to at least a portion of the Fc region of an immunoglobulin. In these fusions, the AMH binding polypeptides form the animo-terminal portion of the fusions, the Fc region forms the carboxy terminal portion of the fusions. The Fc region is preferably limited to the hinge region and the C_H2 and C_H3 domains. The fusion proteins, referred to as an AMH receptor/IgG may be purified from conditioned medium on a Protein A Sepharose column.

The AMH receptor polypeptides are useful in radioreceptor assays to measure all bindable forms of AMH. Screening assays of this kind are conventional in the art and any such screening procedure may be employed, whereby the test sample is contacted with the AMH receptors of this invention and the extent of

binding and biological activity of the bound molecule are determined.

The AMH receptor polypeptides of this invention are useful for purifying molecules that bind to an AMH receptor polypeptide, i.e, the AMH ligand. Such purification comprises contacting a sample containing the AMH ligand to be purified with the AMH receptor polypeptide immobilized on a support under conditions in which the AMH ligand is selectively adsorbed onto the immobilized receptor, washing the immobilized support to remove non-adsorbed material and separating the AMH ligand from the immobilized AMH receptor polypeptide to which it is adsorbed.

The AMH receptor polypeptides of this invention may also be used to induce the formation of anti-AMH-receptor antibodies, which are identified by routine screening. Such antibodies may either be polyclonal or monoclonal antibodies, or antigen binding fragments of such antibodies (such as, for example, F(ab) or $(Fab)_2$ fragments). Of particular significance to the invention are antibodies (and antigen-binding fragments of those antibodies) that bind to the extracellular domain of the AMH receptor polypeptide. The most preferred anti-AMH-receptor antibodies (and antigen-binding fragments thereof) are those capable of binding the receptor in its native conformation on the surface of cells.

Polyclonal antibodies to the AMH receptor polypeptide generally are raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the AMH receptor polypeptide and an adjuvant. It may be useful to conjugate the AMH receptor polypeptide (including fragments containing the target amino acid sequence) to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or

soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, or $R^1N=C=NR$, where R and R^1 are different alkyl groups.

The route and schedule of the host animal or cultured antibody-producing cells therefrom are generally in keeping with established and conventional techniques for antibody stimulation and production. While mice are frequently employed as the test model, it is contemplated that any mammalian subject including human subjects or antibody-producing cells obtained therefrom can be manipulated according to the processes of this invention to serve as the basis for production of mammalian, including human, hybrid cell lines.

Animals are typically immunized against the immunogenic conjugates or derivatives by combining 1 mg or 1 μg of conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with 1/5 to 1/10 the original amount of conjugate in Freund's complete adjuvant (for other suitable adjuvant) by subcutaneous injection at multiple sites. Seven to 14 days later animals are bled and the serum is assayed for anti-AMH receptor polypeptide titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same AMH receptor polypeptide, but conjugated to a different protein and/or through a different cross-linking agent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are used to enhance the immune response.

Monoclonal antibodies are prepared by recovering immune cells, typically spleen cells or lymphocytes from lymph node tissue, from immunized animals and immortalizing the cells in a conventional fashion, e.g., by fusion with myeloma cells or Epstein-Barr virus transformation and screening for clones expressing the desired antibody. The hybridoma technique described originally by Kohler and Milstein, Eur. J. Immunol., 6, p. 511 (1976) and also described by Hammerling et al., Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981) has been widely applied to produce hybrid cell lines that secrete high levels of monoclonal antibodies against many specific antigens. It is possible to fuse cells of one species with another. However, it is preferable that the source of the antibody-producing cells and the myeloma be from the same species.

The hybrid cell lines can be maintained in culture in vitro in cell culture media. The cell lines of this invention can be selected and/or maintained in hypoxanthine-aminopterin thymidine (HAT) medium. In fact, once the hybridoma cell line is established, it can be maintained on a variety of nutritionally adequate media. Moreover, the hybrid cell lines can be stored and preserved in any number of conventional ways, including freezing and storage under liquid nitrogen. Frozen cell lines can be revived and cultured indefinitely with resumed synthesis and secretion of monoclonal antibody.

Monoclonal antibodies may be raised against the extracellular domain of the AMH receptor by immunizing an animal according the above-described procedures with an AMH receptor/IgG fusion protein. Preferably, monoclonal antibodies directed against the extracellular domain will recognize the AMH receptor in its native conformation on the surface of cells and can

be distinguished from those directed against the IgG portion of the fusion protein by standard methods.

Anti-AMH receptor monoclonal antibodies to the extracellular domain of the AMH receptor polypeptide are preferably produced according to the following regime. Animals are first immunized with CHO cells expressing high levels of AMH receptor polypeptides. Prior to fusion of spleen cells with myeloma cells, the animals are boosted with the AMH receptor/IgG fusion protein. Monoclonal antibodies directed against the AMH receptor are identified by standard methods.

The secreted antibody is recovered from tissue culture supernatant by conventional methods such as precipitation, ion exchange chromatography, affinity chromatography, or the like. The antibodies described herein may also be recovered from hybridoma cell cultures by conventional methods for purification of IgG or IgM. The purified antibodies are sterile filtered, and optionally are conjugated with a detectable marker such as an enzyme or spin label for use in diagnostic assays of the AMH-receptor in test samples.

While routinely mouse monoclonal antibodies are used, the invention is not so limited; in fact, human antibodies may be used and may prove to be preferable. Such antibodies can be obtained by using human hybridomas (Cote et al., <u>Monoclonal Antibodies and Cancer Therapy</u>, Alan R. Liss, p. 77 (1985)). In fact, according to the invention, techniques developed for the production of chimeric antibodies (Morrison et al., <u>PNAS</u>, 81, p. 6851 (1984); Neuberger et al., <u>Nature</u>, 312, p. 604 (1984); Takeda et al., <u>Nature</u>, 314, p. 452 (1985); Shaw et al., <u>J. Nat. Canc. Inst.</u>, 80, pp. 1553-1559 (1988); and Oi et al., <u>BioTechniques</u>, 4, p. 214 (1986)) by splicing the genes from a mouse

antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity (such as ability to activate human complement and mediate ADCC) can be used; such antibodies are within the scope of this invention. Also included within the scope of this invention are humanized monoclonal antibodies generated by replacing the complimentary determining regions (CDRs) of a human antibody with the CDRs from an anti-AMH receptor monoclonal antibody.

Techniques for creating recombinant DNA versions of the antigen-binding regions of antibody molecules (known as Fab fragments) which by pass the generation of monoclonal antibodies, are also encompassed within the scope of this invention. This may be accomplished by extracting antibody specific messenger RNA molecules from immune system cells taken from an immunized animal, transcribing these into complementary DNA (cDNA), and cloning the cDNA into a bacterial expression system. One example of such a technique suitable for the practice of this invention incorporates a bacteriophage lambda vector system that contains a leader sequence causing the expressed Fab protein to migrate to the periplasmic space (between the bacterial cell membrane and the cell wall) or to be secreted. One can rapidly generate and screen great numbers of functional Fab fragments for those that bind the antigen. Such AMH-receptor-binding molecules (Fab fragments with specificity for the AMH receptor polypeptide) are specifically encompassed within the term "antibody" as it is defined, discussed, and claimed herein.

The anti-AMH receptor antibodies of the present invention may also be used for diagnostic purposes, such as to measure the expression and function of a patient's AMH receptors. The anti-

receptor antibodies also can be used in imaging to identify and characterize tumors or other tissues, or to define the presence and site of receptor expressing cells.

For diagnostic purposes, the receptors and anti-receptor antibodies can be used in accordance with immunoassay technology. Examples of immunoassays are provided by Wide, Radioimmune Assay Method, Kirkham and Huner, Eds., E & S Livingstone, Edinburgh, pp. 199-206 (1970).

Thus, in one embodiment, AMH receptor polypeptides can be detectably labeled and incubated with a test sample containing AMH molecules, such as biological fluids and the amount of receptor molecule bound to the sample is ascertained. In a second embodiment, antibody to the AMH receptor polypeptides can be used to create a sandwich type immunoassay. In one such assay, a sample suspected of containing AMH can be incubated in the presence of an immobilized anti-AMH antibody. Solubilized, detectably labeled AMH receptor polypeptides are added to the reaction mixture and the amount of AMH is determined by measuring the amount of bound receptor.

As will be appreciated by those of skill in the art, various alternative assays can also be devised. The assay may be merely diagnostic for the presence of AMH or it may be made quantitative by comparing the measure of labeled molecule with that obtained for a standard sample containing known quantities of AMH.

In another diagnostic test suitable for the AMH receptor polypeptides of this invention involves a single incubation step as the antibody (or receptor) bound to the solid support and labeled receptor (or antibody) are both added to the sample being tested at the same time. After the incubation is completed, the

solid support is washed to remove the residue of fluid sample and uncomplexed labeled antibody. The presence of labeled molecules associated with the solid support is then determined as it would be in a conventional sandwich assay.

Antibodies directed against cell surface antigens such as the AMH receptor also have the capacity to specifically target medical therapies against cancers and tumors in tissues expressing the AMH receptor. The anti-AMH antibody may be effective by itself through antibody dependent and complement dependent cytolysis mediated by the Fc domain. Such antibodies can be made more effective as cancer therapeutics by using them as delivery vehicles for drugs, toxins and radionuclides.

One example of an anti-AMH antibody therapy is to conjugate the toxic A chain of ricin or a modified full length form of ricin (which can no longer bind cells) to an antibody directed against the AMH receptor polypeptide expressed on the surface of malignant cells. Such an approach has proved successful with blocked ricin conjugated to a monoclonal antibody against the CD19 antigen expressed on 95% of neoplastic (and normal cells) (Grossbard et al., Blood, 79, p.576 (1992). As will be appreciated by those of skill in the art, other toxins may be equally useful. This approach should prove even more successful using an anti-AMH receptor antibody because the AMH receptor is only expressed in a very limited number of tissues, i.e., the adult gonads.

Another approach to such medical therapies is to use radioisotope labeled anti-AMH antibodies. Such radiolabeled anti-AMH antibodies will preferentially target radioactivity to tumor sites in cells expressing the AMH receptor, sparing normal tissues. Depending on the radioisotope employed, the radiation emitted from a

radiolabeled antibody bound to a tumor cell may also kill nearby malignant cells that do not express the AMH receptor. A variety of radionuclides may be used. Isotopes that emit ß particles (e.g., ^{131}I) have been successful when employed with monoclonal antibodies against CD20 present on B-cell lymphomas (Kaminski et al., N. Engl. J. Med., 329, p. 459 (1993) and Press et al., N. Engl. J. Med., 329, p. 1219 (1993). Radionuclides emitting ß particles generate radioactive emissions that are tumoricidal over distances spanning several cell diameters, permitting the eradication of antigen negative cells and diminishing the consequences of inhomogeneous deposition of antibody in tumors.

Radionuclides emitting α particles may also be employed. The low dose rate irradiation generated by radionuclide labeled anti-AMH antibodies may be more therapeutically effective than the instantaneous irradiation delivered externally in conventional radiation therapy. Low dose rate irradiation can induce apoptosis (programmed cell death) in certain cell lines (Macklis et al., Radiat. Res., 130, p. 220 (1992) and Maklis et al., Radiopharm., 5, p. 339 (1992).

The following procedures for isolating AMH receptor DNA sequences and polypeptides according to this invention are set forth for the purposes of illustration only and are not to be construed as limiting the scope of the invention in any manner. Other methods for isolating or preparing the receptor polypeptides of this invention will be apparent to those of skill in the art.

Isolation and Cloning of the AMH Receptor

To isolate and clone the DNA sequences of this invention, we adopted a selection strategy based upon the TGF-ß receptor family. Accordingly, we used

64-fold degenerate probe encoding the sequence Tyr Met Ala Pro Glu Val [SEQ ID NO: 5], from a highly conserved region within the serine-threonine kinase domain of the TGF-ß type II receptor family to probe a size-selected cDNA library prepared from fetal ovaries. This tissue was chosen for the cDNA library because it responds to AMH by a reduction of aromatase activity. B.Vigier et al., PNAS, 86, pp. 3684-3688 (1989).

Polyadenylated RNA was prepared from 300 ovaries obtained from 22-day-old New Zealand rabbit fetuses. J.Chirgwin et al., Biochemistry, 18, pp. 5294-5299 (1979). Double-stranded cDNA was synthesized using M-MLV reverse transcriptase (Superscript Plasmid System, Gibco-BRL, Gaithersburg, Maryland). After addition of non-palindromic BstXI linkers (Librarian kit, Invitrogen, San Diego, California), the cDNA was size-fractionated on a 1% agarose gel and the fraction containing cDNA above 1.6 kb was ligated into the BstXI site of the plasmid vector pCDM8. The ligated DNA was electroporated into E.coli strain MC1061/P3 (B.Seed and A.Aruffo, PNAS, 84, pp. 3365-3369 (1987) and yielded a library of 2.5 x 10^6 independent clones. The library was subsequently amplified according to standard techniques. From the amplified library, 2 x 10^6 clones were screened on Gene-screen filters (New England Nuclear, Massachusetts) using the ^{32}P-labelled 64-fold degenerate antisense oligonucleotide probe 5' AC(C/T)TC(A/G/C/T)GG(A/G/C/T)GCCAT(A/G)TA 3' [SEQ ID NO: 6] that encodes the Tyr Met Ala Pro Glu Val [SEQ ID NO: 5] peptide. R.Cate et al., Cell, 45, p. 685 (1986). The final wash was at 50°C with 3.2M tetramethylammonium chloride/1% SDS.

Seven hundred positive clones were detected by autoradiography and plated in 96 well plates (Costar, Cambridge, Massachusetts). A second round of screening was performed on nitrocellulose filters which

were inoculated with medium from the 96 well plates using a manifold prong. Positive clones were colony purified, DNA was prepared and subjected to dideoxynucleotide sequencing. (F. Sanger et al., PNAS, 74, 5463-5467 (1977)). The protein sequences translated from the nucleotide sequences of positive clones was compared to the Genebank/EMBL data bases using the TFASTA program. W.Pearson et al., PNAS, 85, pp. 2444-2448 (1988).

Among the positive clones, we identified rabbit cDNAs coding for receptors for several members of the TGF-ß family, including the activin receptor type II (ActR-II) (L.Mathews and W.Vale, Cell, 65, p. 973 (1993)), the TGF-ß receptor type II (TGFßR-II) (H.Lin et al. Cell, 68, p. 775 (1992)), and the type I receptor identified by R.Ebner et al., Science, 260, pp. 1344-1348 (1993) and W.He et al., Developmental Dynamics, 196, pp. 133-142 (1993). One clone, designated 7F2, clearly belonged to the TGF-ß family of receptors but differed from the activin and TGF-ß type II receptors and from the type I receptor reported by He et al. Two additional clones related to 7F2 were identified among the 700 positive clones. These clones, designated 2B10 and 3D6, were completely sequenced.

Proteins encoded by clones 7F2, 2B10 and 3D6 are schematically shown in Figure 1A. The longer clone, 3D6, contains a 183 base pair insertion near its 5' end. We believe that this insertion represents an extra exon because it stays in frame throughout (Figures 1B and 1C) and because 3 of its 4 cysteines are aligned with those of either the TGF-ß or the activin receptor (Figure 1D). The presence of the extra exon in 3D6 suggests that clone 2B10 is generated by alternate splicing. Clone 3D6 has a shorter 3' untranslated region than 2B10 and contains two introns,

A (159 bp) and B (120 bp). The sequence of these clones in the consensus region VIII (Figure 1D) is Tyr Met Ala Pro Glu Leu [SEQ ID NO: 7] instead of the Tyr Met Ala Pro Glu Val [SEQ ID NO: 5] from which we designed the probes used to screen the library.

To obtain a functional full-length clone, we constructed a hybrid clone, designated H1, by joining the extracellular region of 3D6 to the transmembrane kinase region of 2B10 (Figure 1A) at the BspMI restriction site. The homology of H1 with other TGF-ß family receptors was determined according to Kanehisa M. Kanehisa, Nucleic Acids Res., 14, pp. 203-213 (1984) using standard parameters. The intracellular region of the H1 protein exhibits 30.9% homology with human TGFßR-II and 31.9% homology with ActR-II.

Plasmid pB210 is exemplified by a culture depositied in the American Type Culture Collection, Rockville, Maryland on December 16, 1993 and assigned accession number ATCC 69520. Plasmid pH1 is exemplified by a culture deposited in the American Type Culture Collection on December 16, 1993 and assigned accession number ATCC 69521.

Expression Profile of the AMH Receptor

Because AMH exerts its effects solely on the reproductive tract, one would expect its receptor to be expressed only in reproductive organs.

Northern Blot Analysis

A Northern blot analysis of various rabbit tissues at different developmental stages using the following procedures is shown in Figures 2A and 2B.

We obtained various tissues from postnatal male and female rabbits and 22-day-old fetal rabbits. Regression of the male fetal Mullerian duct begins at 20 days post coitum and is significantly advanced at 22 days. Puberty in New Zealand rabbits occurs at

approximately 12 weeks. We prepared Sertoli cells from seminiferous tubules isolated from testicular tissue from a 7-week-old rabbit. The Sertoli cells were purified and cultured as described in B.Vigier et al., Mol. Cell. Endocrinol., 43, pp. 141-150 (1985) except that the Percoll gradient was omitted. Histological examination demonstrated that no germ cells persisted after 5 days in tissue culture.

RNA was isolated as described in J.Chirgwin et al., Biochemistry, 18, pp. 5294-5299 (1979). 10 μg samples were placed in each lane in Figure 2A (except for heart which had 7.65 μg). 20 μg samples were placed in each lane in Figure 2B (except for 4-week-old ovaries which had 18 μg). All RNA samples were electrophoresed on 1% agarose/1% formaldehyde gels, blotted onto Hybond N membranes (Amersham, UK), hybridized as described in R.Cate et al., Cell, 45, pp. 685-698 (1986) with the 1963 bp insert of clone 7F2 removed by digestion with XhoI, and labeled with ^{32}P by random priming (Megaprime labeling kit, Amersham). The blots were exposed 3 days at -80°C with amplifying screens to Kodak XAR film. Size markers were taken from the 0.24-9.5 kb RNA ladder (Gibco-BRL). After hybridization, the blots were stripped and rehybridized with the 1.1 kb PstI fragment of mouse β-actin cDNA. S.Alonso et al., J. Mol. Evol., 23, pp. 11-22 (1986).

In Figure 2A, a 2,350 b band is observed only in the ovary, the testis and the female fetal reproductive tract; a fainter signal is seen in the male fetal reproductive tract whose Mullerian duct has already significantly regressed. This is the expected expression profile of the AMH receptor. The ontogeny of expression in reproductive tissues is shown in Figure 2B. A strong message is observed in the developing and adult ovary but only faintly during pregnancy. In the testis, a comparable message, not

affected by tissue culture-induced loss of germ cells in seminiferous tubules, disappears at adulthood, indicating that it is expressed in immature Sertoli cells.

In Situ Hybridization

To evaluate the specific cells which express the AMH receptor in the reproductive tissues analyzed above, we performed in situ hybridization. The 1963 bp insert of 7F2 was removed by XhoI and cloned at the same site in Bluescript KSII+ plasmid vector (Stratagene, La Jolla, California). ^{35}S-UTP sense and anti-sense RNA probes were produced from T7 and T3 promoters and diluted at 10^5 cpm μl^{-1} in hybridization buffer and the in situ hybridization was performed as described in N.Josso et al., Early Hum. Dev., 33, pp. 91-11 (1993). Slides were exposed 10 days at 4°C. After development, the slides were viewed under dark field illumination x 250.

In situ hybridization (Figure 2C) showed that the message was expressed in the mesenchyme surrounding the Mullerian duct and in granulosa cells of ovarian follicles. In the testis, the message was restricted to seminiferous tubules.

Expression of the Two Receptor Isoforms

We performed reverse-transcriptase polymerase chain reaction (RT-PCR) to determine the relative levels of the H1 and 2B10 mRNAs in AMH target tissues according to the following procedure.

RT-PCR was run with 200 units of M-MLV reverse transcriptase in 40 μl of the buffer supplied by the manufacturer (Gibco-BRL) containing 1.25 mM dNTP, with 5 μg total RNA and 200 pmol random hexanucleotide primer, for 45 minutes at 42°C. A 4 μl aliquot of this reaction product was directly used for PCR amplification, as described in B.Knebelmann et al. PNAS, 88, pp. 3767-3771 (1991) except that magnesium

concentration of the buffer was lowered to 1.34 mM. Twenty pmol of each of the two oligonucleotides, 5' GCAGGATGCT GGGCACTCTG 3' [SEQ ID NO: 8] and 5' GTCAGCACCA CAGGAGCAGG 3' [SEQ ID NO: 9] flanking the extra exon were used (see Figure 4A), in a 100 μl reaction. Amplification was carried out for 30 cycles of denaturation at 95°C for 45 seconds, annealing at 60°C for 45 seconds, and elongation at 72°C for 75 seconds. For control amplification, 50 ng of DNA of clones or 0.5 μg rabbit genomic DNA were used. 15 μl of PCR product were electrophoresed on 12% acrylamide:bis 39:1 gels and stained by ethidium bromide.

As shown in Figure 4, both H1 and 2B10 are expressed in AMH target tissues with a slight prevalence of the H1 isoform. All control tissues are negative except male fetal liver, which expresses low amounts of the shorter 2B10 isoform. The identity of the PCR bands was checked by blot hybridization using labeled internal oligonucleotides indicated by asterisks in Figure 4A. The 5' oligonucleotide, 5' CGCAGGAAGC AGTGCCCAAA 3' [SEQ ID NO: 10] hybridizes with both the 164 and the 347 bp bands. The other oligonucleotide, 5' ACACACAGGT CCTCCTGTTT 3' [SEQ ID NO: 11] hybridizes only with the 347 bp band, specific to the extra exon. The band corresponding to contaminant genomic DNA begins to emerge in cDNA samples which do not contain significant amounts of receptor cDNA(s).

Binding of Iodinated AMH to Clone H1 Expressed in COS cells

To confirm that clones H1 and 2B10 encode the AMH receptor, we introduced these clones into COS cells to determine if the transfected cells bind to AMH. AMH must be cleaved near the C-terminus for bioactivity and

the N-terminal fragment must remain associated with the C-terminal fragment for full activity. C.Wilson et al., Mol. Endocrinol., 7, pp. 247-257 (1993). Therefore, binding studies were carried out with AMH cleaved by plasmin, which generates a non-covalent complex of the N and C-terminal fragments. This complex is extremely sensitive to iodination and loses bioactivity at specific activities above 4 μCi μg^{-1}.

Full-length and plasmin-cleaved AMH preparations (C.Wilson et al., Mol. Endocrinol., 7, pp. 247-257 (1993)) were iodinated by chloramine T oxidation as described in C.Frolik et al., J. Biol. Chem., 259, pp. 10995-11000 (1984). The resulting specific activity was approximately 15-25 μCi μg^{-1} and 1-4 μCi μg^{-1} for full-length and plasmin-cleaved AMH, respectively. The bioactivity of the labeled preparations, checked by the anti-aromatase assay N.di Clemente et al., Development, 114, pp. 721-727 (1992) after sham labeling with cold iodine, was at least 70% of the unlabeled control preparation. COS-M6 cells (3 x 10^5) were plated on either poly-D-lysine-coated one-chambered Labtek microscope slides or 6-well plates (Costar, Cambridge, Massachusetts). Twenty four hours later, cells were transfected by the DEAE/chloroquine procedure as described in A.Aruffo and B.Seed, PNAS, 84, pp. 8573-8577 (1987) except that all reagents were added to the culture medium at the same time. The cells were cultured 3 days in Eagle's minimum essential medium containing 10% female fetal serum and exposed 4 hours at 4°C to iodinated AMH in RPMI medium (Eurobio, Les Ulis, France) containing 0.5% bovine serum albumin. Cells on Labtek slides were exposed to a 1 nM concentration of either full-length or plasmin-cleaved AMH and prepared for autoradiography as described in L.Mathews and W.Vale, Cell, 65, p. 973 (1993). After 10 days, the slides were developed, dehydrated, stained

with toluidine blue and examined under dark field illumination. Transfection efficiency was approximately 30%, as determined by transfection with ß-galactosidase DNA and staining with 5-bromo-4-chloro-3-indolyl-ß-galactopyranoside.

Results of binding studies with labeled AMH preparations are shown in Figure 3. COS cells transfected with clone H1 bind plasmin-cleaved but not full-length AMH; cells transfected with the short receptor isoform 2B10 or with ß-galactosidase DNA do not bind plasmin-cleaved AMH. These results indicate that H1 encodes a receptor that is competent for AMH binding, while the truncated form encoded by 2B10 is not.

To assure that these results were statistically significant, transfected cells (prepared as described above) cultured in 6-well dishes were exposed to 0.5, 1, or 2 nM iodinated plasmin-cleaved AMH, with or without a 100-fold excess of the cold ligand as described in L.Mathews and W.Vale, Cell, 65, p. 973 (1993) and displaceable binding was measured. The results are recorded in Table 1.

Table 1

AMH conc. (nM)	sp act µCi/µg	displaceable counts per min H1	2B10	3F11*
0.5	2.6	1898		1343
0.5	4.1	1846	133	570
1.0	1.0	306		121
1.0	1.0	228		-56
1.0	1.0	516		17
1.0	2.6	2073	1527	970
1.0	4.1	2992	1692	2457
2.0	2.6	3496	1681	2480

* 3F11 is full length cDNA of rabbit TGFßR-II

Displaceable counts represent the difference between mean counts bound to cells exposed only to

labeled plasmin-cleaved AMH and that of counts bound to cells exposed to both labeled and a 100-fold excess of cold ligand, each determined in triplicate. The number of counts corresponding to 1 nM of labeled ligand varied between 150,000 and 1,260,000 cpm, according to the specific activity. In the four instances where the three clones were tested in the same experiment, Student's paired t test analysis shows no significant difference between displaceable binding to clones 2B10 or 3F11 ($p=0.337$). In contrast, displaceable binding to clone H1 is significantly higher than that to either 2B10 ($p=0.019$) or 3F11 ($p=0.008$) transfected cells. When H1 was compared to either 2B10 or 3F11 ($n=12$), the difference was even more striking ($p< 0.001$).

Isolation of the Human AMH Receptor cDNA

To isolate the cDNA for the human AMH receptor, we screened a human testis cDNA library with a DNA probe derived from the rabbit AMH receptor cDNA clone H1. The human testis library was made from RNA isolated from human testis obtained from a 6 month old patient with androgen insensitivity. Chirgwin, Biochemistry, 18, pp. 5294-5299 (1979). Double stranded cDNA was synthesized from polyadenylated testis RNA using the Time Saver kit from Pharmacia. After addition of EcoR1/Not1 linkers, the cDNA was size selected and ligated into the EcoR1 site of λgt11. Aliquots of the ligation were packaged into phage particles using Gigapack II (Stratagene). The packaged DNA was used to infect E. coli 1090 cells. Plating of the library yielded 2.6×10^6 independent plaques and was subsequently amplified. The titre of the phage library was 8.5×10^{10} pfu/ml.

We screened the library with a random primed probe derived from the rabbit AMH receptor cDNA clone H1, using the plaque hybridization screening technique

of Benton and Davis (Science, 196, p. 180 (1977)). 2 X 10^6 plaques were screened on Hybond N (Amersham) filters with the ^{32}P-labeled probe. The 2494 bp probe was derived from clone H1 by digesting plasmid H1 with Xho1, purified on an agarose gel, and ^{32}P-labeled using the random priming method (Megaprime labeling kit, Amersham). Conditions were standard, and the final washing conditions were 2 X SSC, 0.1% SDS at 55°. We detected positive hybridizing clones by autoradiography. These plaques were isolated and rescreened at lower density, until completely pure.

DNA was purified from one of the positive clones, designated λ-hAMHR-3. The insert was removed with EcoR1 and cloned into the EcoR1 site of plasmid Bluescript KS II (+). The resulting plasmid was designated KS-hAMHR3-2. The insert was completely sequenced by the method of Sanger et al. and is shown in SEQ ID NO: 12. A comparison of the predicted amino acid sequence with that of the rabbit AMH receptor is shown in Figure 5 [SEQ ID NO: 4 AND SEQ ID NO 13]. The two proteins share 82% similarity, indicating that clone 3-2 encodes the human AMH receptor.

Plasmid hAMHR3-2 is exemplified by a culture depositied in the American Type Culture Collection, Rockville, Maryland on December 13, 1994 and assigned accession number ATCC ________.

Genetic Evidence that Clone 3-2 Encodes the Human AMH Receptor

In order to prove that clone 3-2 encodes the human AMH receptor, we analyzed the gene that encodes the 3-2 cDNA in normal humans and in humans who suffer from possible mutations in the AMH receptor. These males are externally virilized but retain a uterus and fallopian tubes, a condition termed Persistent Mullerian duct syndrome (PMDS). It has been shown that

in some of these patients, the gene for AMH contains mutations, rendering the AMH that is produced nonfunctional. Imbeaud et al., Hum. Mol. Genet., 3, pp. 125-131 (1994). This accounts for the persistence of the Mullerian duct in these individuals, since no functional AMH is present to cause the regression of the Mullerian duct during fetal development. However, some individuals that suffer from PMDS have completely normal AMH, indicating that their AMH receptor may be nonfunctional due to mutations. Thus we wanted to analyze the gene that encodes the 3-2 cDNA in such individuals to see if they did indeed contain a mutation.

A portion of the gene was analyzed in a 2.6 year old patient (Patient T.A.) with AMH positive (i.e. functional AMH could be detected in a testicular biopsy sample) PMDS. Single strand conformational polymorphism (SSCP) analysis [Orita et al., PNAS, 86, pp. 2766-2770 (1989)] was performed on PCR products generated from DNA isolated from lymphocytes obtained from patient T.A. and from a normal human male, using primers designed from the 3-2 cDNA clone sequence. This analysis allows the detection of single base changes. The SSCP analysis detected a polymorphism in a portion of the gene that encodes the extracellular domain of the receptor. Two PCR primers (1s [SEQ ID NO: 15] and 2a [SEQ ID NO: 16]) were then used to amplify the portion of the gene which contained this polymorphism. The PCR product was cloned into the pGEM-T cloning vector using the AT cloning method of Promega and sequenced. The sequence is shown in Figure 6 [nucleotides 401-800 of SEQ ID NO: 14]. The sequence covers an exon from the extracellular domain and an adjacent intron. At the junction of the exon and intron, one finds the dinucleotide sequence AT, instead of the GT dinucleotide sequence found at the 5'

end all introns, indicating that the receptor gene in patient T.A. contains a splicing mutation.

To confirm that this change is indeed a mutation, we cloned a portion of the gene from a human genomic λEMBL4 library. This library was made with genomic DNA isolated from normal human muscle, thus any 3-2 receptor gene isolated from this library should have a normal (i.e. wild type) sequence. The gene was cloned from the λEMBL4 library using standard conditions, and using the insert from clone 3-2 as a probe. A clone (51bb) containing the 5' portion of the gene was isolated, DNA was purified, and a 8 kb EcoR1 fragment was excised and subcloned into the vector Bluescript KS II (+) to generate clone 3-35. A partial nucleotide sequence derived from clone 3-35 is shown in Figure 7 [SEQ ID NO: 14]. It covers the first two exons and a portion of the third exon, all of which are within the extracellular domain of the receptor. The sequence also contains two introns, shown in lower case. Both introns begin with the dinucleotide GT and end with the dinucleotide AG. It is the GT dinculeotide in the second intron shown in Figure 7 [SEQ ID NO: 14], which has been mutated in the gene of patient T.A.

Furthermore, both receptor genes of patient T.A. contain this mutation, which is consistent with the fact that PMDS is an autosomal recessive genetic disease (i.e. the function of both genes must be eliminated in order to see a phenotype). The G>A mutation destroys an Hph 1 site; thus one can assess the presence of the mutation by digesting the PCR fragment (generated with primers 1s [SEQ ID NO: 15] and 2a [SEQ ID NO: 16]) with Hph 1. The PCR fragment made from normal DNA was completely digested with Hph 1, while the PCR fragment made from patient T.A. DNA was resistant to digestion with Hph 1, indicating that both

alleles contain the G>A mutation. This implies that the patient inherited one mutant gene from each parent. Indeed, an Hph 1 digestion of the PCR fragment made from the DNA of the mother and father indicated that only 50% of the fragment could be digested (i.e. both the mother and father have one normal gene and one mutant gene).

In order to demonstrate that the mutant gene causes a problem with splicing, RNAs isolated from a testicular biopsy of patient T.A. and from normal human fetal testis were subjected to RT-PCR using primers 1s [SEQ ID NO: 15] and 3a [SEQ ID NO: 17]. The PCR products were analyzed by agarose gel electrophoresis (Figure 8); they were also cloned into the pGEM-T vector using the AT cloning method of Promega and sequenced. Only one fragment was produced with RNA from the normal samples, while two variant PCR products were produced from patient T.A. RNA (Figure 8). The large fragment was shown by sequencing to contain a portion of the intron (12 bp), reflecting an aberrant mRNA that was generated by use of a cryptic splice donor site downstream of the mutated donor site (Figure 9). The smaller variant PCR fragment was shown by sequencing to be missing the second exon; this product reflects an mRNA that has undergone exon skipping. The smaller mRNA would be expected to produce a non-functional receptor, since it is identical to the alternately spliced receptor (2B10) produced in the rabbit, which we have shown cannot bind AMH. The mRNA that is produced by cryptic splicing would contain a 4 amino acid insertion in the middle of the extracellular domain, and would also be expected to be non-functional.

Therefore, the presence of a splicing mutation in both copies of the 3-2 gene in patient T.A. who

suffers from PMDS, confirms that the 3-2 gene encodes the AMH receptor.

Expression of the AMH receptor in a Tumor Sample

To demonstrate that the AMH receptor can be used to direct a toxin to a tumor cell, it is necessary to show that tumors express the AMH receptor. We have used RT-PCR to show that four granulosa cell tumors do express the AMH receptor. RNA was isolated from the tumor samples and subjected to RT-PCR using two primers (1s [SEQ ID NO: 15] and 2a [SEQ ID NO: 16]) from the human AMH receptor cDNA sequence. As shown in Figure 10, a PCR product of the expected size is generated from the RNA of these tumor samples.

Therefore, a toxin coupled to an antibody against the AMH receptor can be used to eradicate these tumors.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: BIOGEN, INC.
CATE, Richard L.
INSERM, (U.293)
JOSSO, Nathalie

(ii) TITLE OF INVENTION: ANTI-MULLERIAN HORMONE RECEPTOR POLYPEPTIDES AND ANTIBODIES THERETO

(iii) NUMBER OF SEQUENCES: 17

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: c/o FISH & NEAVE
(B) STREET: 1251 Avenue of the Americas
(C) CITY: New York
(D) STATE: New York
(E) COUNTRY: U.S.A.
(F) ZIP: 10020

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/166,333
(B) FILING DATE: 13-DEC-1993

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/173,512
(B) FILING DATE: 23-DEC-1993

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Haley Jr., James F.
(B) REGISTRATION NUMBER: 27,794
(C) REFERENCE/DOCKET NUMBER: B174CIP

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (212) 596-9000
(B) TELEFAX: (212) 596-9090

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2228 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..2228
(D) OTHER INFORMATION: /note= "clone 2B10"

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 62..112

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 113..1585

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 113..310
(D) OTHER INFORMATION: /note= "extracellular domain"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 311..388
(D) OTHER INFORMATION: /note= "transmembrane region"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 389..1585
(D) OTHER INFORMATION: /note= "cytoplasmic domain"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 200..205
(D) OTHER INFORMATION: /note= "BspM1 restriction site"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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AAAGGGAGCG CTGGCCGCGC TGGCTGGTGC TCTCCTGCAG CGGCTGCCAC CCTGCGGCAG    60

GATGCTGGGC ACTCTGGGGC TTTGGGCACT GCTTCCTGCG GCTGTGCAGG GATGCCGAGA   120

CAGTGACGAG CCAGGCTGTG AGTCCCTTAG CTGTGACCCG AGCCCCCGAG CCCGCGCCAG   180

CTCTGGCTCC ACTCTCTTCA CCTGCTCCTG TGGTGCTGAC TTCTGCAATG CCAACTACAG   240

CCATCTGCCT CCTCTGGGGG GCCCTGGGAC TCCTGGCCCC CAGGGTCCCC AGGCTGCCCC   300
```

AGGTGAGTCC CCCTGGATGG CACTGGCGCT GCTGGGGCTG GTCCTGCTGC TGCTGCTGCT 360

GCTGGGCGGT ATTGTCGTGG CTCTGCTCCA GCGAAAGGCC TACAGGGTGC AGAGTGGGCC 420

AGAGCCAGAG CCAGACTCAG GCAGGGACTG CAGTGAGGAG CTGCCGGAGC TGCCCCAGCT 480

GTGCTTCTCC CAGGTCATCC GGGAAGGAGG TCACGCGGCA GTGTGGGCTG GGCAGCTGCA 540

AGGGGAGCTG GTGGCCATCA AGGTCTTCCC CCGGAGGGCC GTGGCCCAGT TCCGAGCTGA 600

GAGAGCCTTG TATGAGCTGC CGGGCCTGCA GCACAACCAC GTTGTCCGAT TTATCGCTGC 660

TGGCCAGGGG GGACCCGGCC CCCTGCCCTC TGGGCCCCTG CTGGTACTGG AACTGCACCC 720

CAAGGGCTCC CTGTGCCAGT ACCTGAGCCA GCACACCAGT GACTGGGGAA GTTCCCTGAG 780

GATGGCTCTG TCTTTAGCCC AGGGCCTGGC ATTTCTCCAT GAGGAGCGCT GGCAGGATGG 840

CCAGTACAAG CCTGGTATTG CCCACCGAGA TCTGAGCAGC CAGAATGTGC TCATCCGGGA 900

AGATGGGTCA TGTGCCATTG GAGACCTGGG CCTGGCCTTG GTGCTCCCTG GTTTCGCTCA 960

GCCCCGTGCC TGGGCCCCTC CGCAGCCCCG AGGCCCAGCG GCCATCATGG AGGCCGGCAC 1020

ACAGAGGTAC ATGGCGCCAG AGCTCTTGGA CAAGTCTCTG GACCTACAGG ACTGGGGCAC 1080

TGCCCTCCGG CGAGCCGACG TCTACTCCTT GGCCCTGCTC CTGTGGGAGA TCCTGAGCCG 1140

CTGCCCCGAT TTGAGGCCTG ACGGCAGACC ACCACCCTTC CAACTGGCCT ATGAAGCAGA 1200

ACTGGGCAGC GCCCCCACCA CCTGTGAGCT GTGGGCCCTG GCAGTAGAGG AGAGGAGGCG 1260

CCCTGACATC CCATCCTCCT GGTGCTGCTT TGCCACAGAC CCCGGCGGGC TCAGGGAGCT 1320

GCTGGAAGAC TGCTGGGACG CAGACCCGGA AGCGCGGCTG ACGGCCGAGT GTGTCCAGCA 1380

GCGCCTGGTG GCCCTGGTTC ATCCTCAGGA GGCCCAGCCC TGCCCAGAGG GCCGTCCACA 1440

CAGCCACCCA GAAGACTGGC CCCCTGCTCC TGCCCCTGCC CCTGCTCTCC TCCCCGGCAG 1500

CCCACAGCCG GGTGCCTGCC ACTTCGGTGT TCAGCAAGGC CTTTGCTCCA GGAACCCCGG 1560

AGCTGCCTGT GCCAGTTCTG ACGTGTAAAT AAGCAGTTTG TGTGTAATCT ACCTGTAAAC 1620

GTAAACATGG CACTGGTATA CCTGTCTGCT CTGCCTCTCC ACTGTTTTCC CACTCCCCAA 1680

TCTGTTAGGC AGGAAGCTGG AAATTGAGCC TATGTGTGTG TGTGTGAGAC AGGCCTGAAG 1740

ACCAGCACCC ATTGCAGAAG CCCCAGAAAC CCAGCATCTT GCACTTCAGA GTCCTGCCCA 1800

GACCCTGCTA ACCTCCCAGG TGGTCCCAGC CCTTCCCTCA AGGCAAACTC CCAGGAGAAT 1860

TCTCTCTCCT CAGGACCAAA GGGGTTACCT GATCTGATAA CACAGGGCAT AAAACCTTCA 1920

CAGAGTCCCC TTGCCAGCAA ACCTGGGGAG GAATTTGCAA ATTTTCACTC AACAAACCCT 1980

TCCACCAGGG CTCCCCCTTC TTTGTCCTGG AGGAGAAAGG GAGGTGGGTA AACAGACTCC 2040

CTTAAAAACC TAGGGAGTCC AAACTGACCA CGCACTCAGC CCTCTGCCTC TCTGCCTAGC 2100

CGCCCGCCTG GCCTGCCCAG GTGTATTCTC TTCATTCAAC CATGTGACCT TGCTCTTCCC 2160

CTGAGCGCTC TCTGTCTGTC CCCTCCGTTT TGACAGATGC CCTGTCCCCA ATAAACCTTA 2220

TCACTCTG 2228

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2408 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..2408
(D) OTHER INFORMATION: /note= "clone H1"

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 59..109

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 110..1765

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 110..490
(D) OTHER INFORMATION: /note= "extracellular domain"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 491..568
(D) OTHER INFORMATION: /note= "transmembrane region"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 569..1765
(D) OTHER INFORMATION: /note= "cytoplasmic domain"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 108..290
(D) OTHER INFORMATION: /note= "extra exon missing in 2B10"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 380..385
(D) OTHER INFORMATION: /note= "BspM1 restriction site"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 2390..2395
(D) OTHER INFORMATION: /note= "poly A signal sequence"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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GGGAGCGCTG GCCGCGCTGG CTGGTGCTCT CCTGCAGCGG CTGCCACCCT GCGGCAGGAT     60
GCTGGGCACT CTGGGGCTTT GGGCACTGCT TCCTGCGGCT GTGCAGGCAC CCCCAAACAG    120
GAGGACCTGT GTGTTCTTTG AGGCCCCTGG AGTGCGGGGA AGCACAAAGA CACTGGGGGA    180
GCTGCTAGAT GCAGGACCAG GGCCCCCCAG GGTTATCCGC TGCCTCTACA GCCGCTGCTG    240
CTTTGGGATC TGGAACCTAA CCCGAGACCA GGCACAGGTG GAGATGCAAG GATGCCGAGA    300
CAGTGACGAG CCAGGCTGTG AGTCCCTTAG CTGTGACCCG AGCCCCCGAG CCCGCGCCAG    360
CTCTGGCTCC ACTCTCTTCA CCTGCTCCTG TGGTGCTGAC TTCTGCAATG CCAACTACAG    420
CCATCTGCCT CCTCTGGGGG GCCCTGGGAC TCCTGGCCCC CAGGGTCCCC AGGCTGCCCC    480
AGGTGAGTCC CCCTGGATGG CACTGGCGCT GCTGGGGCTG GTCCTGCTGC TGCTGCTGCT    540
GCTGGGCGGT ATTGTCGTGG CTCTGCTCCA GCGAAAGGCC TACAGGGTGC AGAGTGGGCC    600
AGAGCCAGAG CCAGACTCAG GCAGGGACTG CAGTGAGGAG CTGCCGGAGC TGCCCCAGCT    660
GTGCTTCTCC CAGGTCATCC GGGAAGGAGG TCACGCGGCA GTGTGGGCTG GGCAGCTGCA    720
AGGGGAGCTG GTGGCCATCA AGGTCTTCCC CCGGAGGGCC GTGGCCCAGT TCCGAGCTGA    780
GAGAGCCTTG TATGAGCTGC CGGGCCTGCA GCACAACCAC GTTGTCCGAT TTATCGCTGC    840
TGGCCAGGGG GGACCCGGCC CCCTGCCCTC TGGGCCCCTG CTGGTACTGG AACTGCACCC    900
CAAGGGCTCC CTGTGCCAGT ACCTGAGCCA GCACACCAGT GACTGGGGAA GTTCCCTGAG    960
GATGGCTCTG TCTTTAGCCC AGGGCCTGGC ATTTCTCCAT GAGGAGCGCT GGCAGGATGG   1020
CCAGTACAAG CCTGGTATTG CCCACCGAGA TCTGAGCAGC CAGAATGTGC TCATCCGGGA   1080
AGATGGGTCA TGTGCCATTG GAGACCTGGG CCTGGCCTTG GTGCTCCCTG GTTTCGCTCA   1140
```

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GCCCCGTGCC TGGGCCCCTC CGCAGCCCCG AGGCCCAGCG GCCATCATGG AGGCCGGCAC   1200
ACAGAGGTAC ATGGCGCCAG AGCTCTTGGA CAAGTCTCTG GACCTACAGG ACTGGGGCAC   1260
TGCCCTCCGG CGAGCCGACG TCTACTCCTT GGCCCTGCTC CTGTGGGAGA TCCTGAGCCG   1320
CTGCCCCGAT TTGAGGCCTG ACGGCAGACC ACCACCCTTC CAACTGGCCT ATGAAGCAGA   1380
ACTGGGCAGC GCCCCCACCA CCTGTGAGCT GTGGGCCCTG GCAGTAGAGG AGAGGAGGCG   1440
CCCTGACATC CCATCCTCCT GGTGCTGCTT TGCCACAGAC CCCGGCGGGC TCAGGGAGCT   1500
GCTGGAAGAC TGCTGGGACG CAGACCCGGA AGCGCGGCTG ACGGCCGAGT GTGTCCAGCA   1560
GCGCCTGGTG GCCCTGGTTC ATCCTCAGGA GGCCCAGCCC TGCCCAGAGG GCCGTCCACA   1620
CAGCCACCCA GAAGACTGGC CCCCTGCTCC TGCCCCTGCC CCTGCTCTCC TCCCCGGCAG   1680
CCCACAGCCG GGTGCCTGCC ACTTCGGTGT TCAGCAAGGC CTTTGCTCCA GGAACCCCGG   1740
AGCTGCCTGT GCCAGTTCTG ACGTGTAAAT AAGCAGTTTG TGTGTAATCT ACCTGTAAAC   1800
GTAAACATGG CACTGGTATA CCTGTCTGCT CTGCCTCTCC ACTGTTTTCC CACTCCCCAA   1860
TCTGTTAGGC AGGAAGCTGG AAATTGAGCC TATGTGTGTG TGTGTGAGAC AGGCCTGAAG   1920
ACCAGCACCC ATTGCAGAAG CCCCAGAAAC CCAGCATCTT GCACTTCAGA GTCCTGCCCA   1980
GACCCTGCTA ACCTCCCAGG TGGTCCCAGC CCTTCCCTCA AGGCAAACTC CCAGGAGAAT   2040
TCTCTCTCCT CAGGACCAAA GGGGTTACCT GATCTGATAA CACAGGGCAT AAAACCTTCA   2100
CAGAGTCCCC TTGCCAGCAA ACCTGGGGAG GAATTTGCAA ATTTTCACTC AACAAACCCT   2160
TCCACCAGGG CTCCCCCTTC TTTGTCCTGG AGGAGAAAGG GAGGTGGGTA AACAGACTCC   2220
CTTAAAAACC TAGGGAGTCC AAACTGACCA CGCACTCAGC CCTCTGCCTC TCTGCCTAGC   2280
CGCCCGCCTG GCCTGCCCAG GTGTATTCTC TTCATTCAAC CATGTGACCT TGCTCTTCCC   2340
CTGAGCGCTC TCTGTCTGTC CCCTCCGTTT TGACAGATGC CCTGTCCCCA ATAAACCTTA   2400
TCACTCTG                                                            2408
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 508 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Domain
(B) LOCATION: 18..83

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 18..508

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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Met Leu Gly Thr Leu Gly Leu Trp Ala Leu Leu Pro Ala Ala Val Gln
1               5                   10                  15

Gly Cys Arg Asp Ser Asp Glu Pro Gly Cys Glu Ser Leu Ser Cys Asp
            20                  25                  30

Pro Ser Pro Arg Ala Arg Ala Ser Ser Gly Ser Thr Leu Phe Thr Cys
        35                  40                  45

Ser Cys Gly Ala Asp Phe Cys Asn Ala Asn Tyr Ser His Leu Pro Pro
    50                  55                  60

Leu Gly Gly Pro Gly Thr Pro Gly Pro Gln Gly Pro Gln Ala Ala Pro
65                  70                  75                  80

Gly Glu Ser Pro Trp Met Ala Leu Ala Leu Leu Gly Leu Val Leu Leu
                85                  90                  95

Leu Leu Leu Leu Leu Gly Gly Ile Val Val Ala Leu Leu Gln Arg Lys
            100                 105                 110

Ala Tyr Arg Val Gln Ser Gly Pro Glu Pro Glu Pro Asp Ser Gly Arg
        115                 120                 125

Asp Cys Ser Glu Glu Leu Pro Glu Leu Pro Gln Leu Cys Phe Ser Gln
    130                 135                 140

Val Ile Arg Glu Gly Gly His Ala Ala Val Trp Ala Gly Gln Leu Gln
145                 150                 155                 160

Gly Glu Leu Val Ala Ile Lys Val Phe Pro Arg Arg Ala Val Ala Gln
                165                 170                 175

Phe Arg Ala Glu Arg Ala Leu Tyr Glu Leu Pro Gly Leu Gln His Asn
            180                 185                 190

His Val Val Arg Phe Ile Ala Ala Gly Gln Gly Gly Pro Gly Pro Leu
        195                 200                 205

Pro Ser Gly Pro Leu Leu Val Leu Glu Leu His Pro Lys Gly Ser Leu
    210                 215                 220
```

Cys Gln Tyr Leu Ser Gln His Thr Ser Asp Trp Gly Ser Ser Leu Arg
225 230 235 240

Met Ala Leu Ser Leu Ala Gln Gly Leu Ala Phe Leu His Glu Glu Arg
245 250 255

Trp Gln Asp Gly Gln Tyr Lys Pro Gly Ile Ala His Arg Asp Leu Ser
260 265 270

Ser Gln Asn Val Leu Ile Arg Glu Asp Gly Ser Cys Ala Ile Gly Asp
275 280 285

Leu Gly Leu Ala Leu Val Leu Pro Gly Phe Ala Gln Pro Arg Ala Trp
290 295 300

Ala Pro Pro Gln Pro Arg Gly Pro Ala Ala Ile Met Glu Ala Gly Thr
305 310 315 320

Gln Arg Tyr Met Ala Pro Glu Leu Leu Asp Lys Ser Leu Asp Leu Gln
325 330 335

Asp Trp Gly Thr Ala Leu Arg Arg Ala Asp Val Tyr Ser Leu Ala Leu
340 345 350

Leu Leu Trp Glu Ile Leu Ser Arg Cys Pro Asp Leu Arg Pro Asp Gly
355 360 365

Arg Pro Pro Pro Phe Gln Leu Ala Tyr Glu Ala Glu Leu Gly Ser Ala
370 375 380

Pro Thr Thr Cys Glu Leu Trp Ala Leu Ala Val Glu Glu Arg Arg Arg
385 390 395 400

Pro Asp Ile Pro Ser Ser Trp Cys Cys Phe Ala Thr Asp Pro Gly Gly
405 410 415

Leu Arg Glu Leu Leu Glu Asp Cys Trp Asp Ala Asp Pro Glu Ala Arg
420 425 430

Leu Thr Ala Glu Cys Val Gln Gln Arg Leu Val Ala Leu Val His Pro
435 440 445

Gln Glu Ala Gln Pro Cys Pro Glu Gly Arg Pro His Ser His Pro Glu
450 455 460

Asp Trp Pro Pro Ala Pro Ala Pro Ala Pro Ala Leu Leu Pro Gly Ser
465 470 475 480

Pro Gln Pro Gly Ala Cys His Phe Gly Val Gln Gln Gly Leu Cys Ser
485 490 495

Arg Asn Pro Gly Ala Ala Cys Ala Ser Ser Asp Val
500 505

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 569 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Domain
(B) LOCATION: 18..144

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 18..569

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Met Leu Gly Thr Leu Gly Leu Trp Ala Leu Leu Pro Ala Ala Val Gln
1               5                   10                  15

Ala Pro Pro Asn Arg Arg Thr Cys Val Phe Phe Glu Ala Pro Gly Val
            20                  25                  30

Arg Gly Ser Thr Lys Thr Leu Gly Glu Leu Leu Asp Ala Gly Pro Gly
        35                  40                  45

Pro Pro Arg Val Ile Arg Cys Leu Tyr Ser Arg Cys Cys Phe Gly Ile
    50                  55                  60

Trp Asn Leu Thr Arg Asp Gln Ala Gln Val Glu Met Gln Gly Cys Arg
65                  70                  75                  80

Asp Ser Asp Glu Pro Gly Cys Glu Ser Leu Ser Cys Asp Pro Ser Pro
                85                  90                  95

Arg Ala Arg Ala Ser Ser Gly Ser Thr Leu Phe Thr Cys Ser Cys Gly
            100                 105                 110

Ala Asp Phe Cys Asn Ala Asn Tyr Ser His Leu Pro Pro Leu Gly Gly
        115                 120                 125

Pro Gly Thr Pro Gly Pro Gln Gly Pro Gln Ala Ala Pro Gly Glu Ser
    130                 135                 140

Pro Trp Met Ala Leu Ala Leu Leu Gly Leu Val Leu Leu Leu Leu Leu
145                 150                 155                 160

Leu Leu Gly Gly Ile Val Val Ala Leu Leu Gln Arg Lys Ala Tyr Arg
                165                 170                 175

Val Gln Ser Gly Pro Glu Pro Glu Pro Asp Ser Gly Arg Asp Cys Ser
            180                 185                 190
```

Glu Glu Leu Pro Glu Leu Pro Gln Leu Cys Phe Ser Gln Val Ile Arg
195 200 205

Glu Gly Gly His Ala Ala Val Trp Ala Gly Gln Leu Gln Gly Glu Leu
210 215 220

Val Ala Ile Lys Val Phe Pro Arg Arg Ala Val Ala Gln Phe Arg Ala
225 230 235 240

Glu Arg Ala Leu Tyr Glu Leu Pro Gly Leu Gln His Asn His Val Val
245 250 255

Arg Phe Ile Ala Ala Gly Gln Gly Gly Pro Gly Pro Leu Pro Ser Gly
260 265 270

Pro Leu Leu Val Leu Glu Leu His Pro Lys Gly Ser Leu Cys Gln Tyr
275 280 285

Leu Ser Gln His Thr Ser Asp Trp Gly Ser Ser Leu Arg Met Ala Leu
290 295 300

Ser Leu Ala Gln Gly Leu Ala Phe Leu His Glu Glu Arg Trp Gln Asp
305 310 315 320

Gly Gln Tyr Lys Pro Gly Ile Ala His Arg Asp Leu Ser Ser Gln Asn
325 330 335

Val Leu Ile Arg Glu Asp Gly Ser Cys Ala Ile Gly Asp Leu Gly Leu
340 345 350

Ala Leu Val Leu Pro Gly Phe Ala Gln Pro Arg Ala Trp Ala Pro Pro
355 360 365

Gln Pro Arg Gly Pro Ala Ala Ile Met Glu Ala Gly Thr Gln Arg Tyr
370 375 380

Met Ala Pro Glu Leu Leu Asp Lys Ser Leu Asp Leu Gln Asp Trp Gly
385 390 395 400

Thr Ala Leu Arg Arg Ala Asp Val Tyr Ser Leu Ala Leu Leu Leu Trp
405 410 415

Glu Ile Leu Ser Arg Cys Pro Asp Leu Arg Pro Asp Gly Arg Pro Pro
420 425 430

Pro Phe Gln Leu Ala Tyr Glu Ala Glu Leu Gly Ser Ala Pro Thr Thr
435 440 445

Cys Glu Leu Trp Ala Leu Ala Val Glu Glu Arg Arg Arg Pro Asp Ile
450 455 460

Pro Ser Ser Trp Cys Cys Phe Ala Thr Asp Pro Gly Gly Leu Arg Glu
465 470 475 480

Leu Leu Glu Asp Cys Trp Asp Ala Asp Pro Glu Ala Arg Leu Thr Ala
485 490 495

Glu Cys Val Gln Gln Arg Leu Val Ala Leu Val His Pro Gln Glu Ala
500 505 510

Gln Pro Cys Pro Glu Gly Arg Pro His Ser His Pro Glu Asp Trp Pro
515 520 525

Pro Ala Pro Ala Pro Ala Pro Ala Leu Leu Pro Gly Ser Pro Gln Pro
530 535 540

Gly Ala Cys His Phe Gly Val Gln Gln Gly Leu Cys Ser Arg Asn Pro
545 550 555 560

Gly Ala Ala Cys Ala Ser Ser Asp Val
565

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Tyr Met Ala Pro Glu Val
1 5

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ACYTCNGGNG CCATRTA 17

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Tyr Met Ala Pro Glu Leu
1 5

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GCAGGATGCT GGGCACTCTG 20

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GTCAGCACCA CAGGAGCAGG 20

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CGCAGGAAGC AGTGCCCAAA 20

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ACACACAGGT CCTCCTGTTT 20

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1833 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..1833
(D) OTHER INFORMATION: /note= "clone 3-2"

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 61..111
(D) OTHER INFORMATION: /note= "putative signal sequence"

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 112..1779

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 112..492
(D) OTHER INFORMATION: /note= "extracellular domain"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 493..570
(D) OTHER INFORMATION: /note= "transmembrane region"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 571..1779
(D) OTHER INFORMATION: /note= "cytoplasmic domain"

(ix) FEATURE:
(A) NAME/KEY: 5'UTR
(B) LOCATION: 1..60

(ix) FEATURE:
(A) NAME/KEY: 3'UTR
(B) LOCATION: 1780..1833

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

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CCAGGGGCAG CTGTGCTGGC TTATGCTCTT CTCCTTCTGC TGCTGCCATC CTCCAGCAAG     60

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AGGCGAACCT GTGTGTTCTT TGAGGCCCCT GGAGTGCGGG GAAGCACAAA GACACTGGGA    180

GAGCTGCTAG ATACAGGCAC AGAGCTCCCC AGAGCTATCC GCTGCCTCTA CAGCCGCTGC    240

TGCTTTGGGA TCTGGAACCT GACCCAAGAC CGGGCACAGG TGGAAATGCA AGGATGCCGA    300

GACAGTGATG AGCCAGGCTG TGAGTCCCTC CACTGTGACC CAAGTCCCCG AGCCCACCCC    360

AGCCCTGGCT CCACTCTCTT CACCTGCTCC TGTGGCACTG ACTTCTGCAA TGCCAATTAC    420

AGCCATCTGC CTCCTCCAGG GAGCCCTGGG ACTCCTGGCT CCCAGGGTCC CCAGGCTGCC    480

CCAGGTGAGT CCATCTGGAT GGCACTGGTG CTGCTGGGGC TGTTCCTCCT CCTCCTGCTG    540

CTGCTGGGCA GCATCATCTT GGCCCTGCTA CAGCGAAAGA ACTACAGAGT GCGAGGTGAG    600

CCAGTGCCAG AGCCAAGGCC AGACTCAGGC AGGGACTGGA GTGTGGAGCT GCAGGAGCTG    660

CCTGAGCTGT GTTTCTCCCA GGTAATCCGG GAAGGAGGTC ATGCAGTGGT TTGGGCCGGG    720

CAGCTGCAAG GAAAACTGGT TGCCATCAAG GCCTTCCCAC CGAGGTCTGT GGCTCAGTTC    780

CAAGCTGAGA GAGCATTGTA CGAACTTCCA GGCCTACAGC ACGACCACAT TGTCCGATTT    840

ATCACTGCCA GCCGGGGGGG TCCTGGCCGC CTGCTCTCTG GGCCCCTGCT GGTACTGGAA    900

CTGCATCCCA AGGGCTCCCT GTGCCACTAC TTGACCCAGT ACACCAGTGA CTGGGGAAGT    960

TCCCTGCGGA TGGCACTGTC CCTGGCCCAG GGCCTGGCAT TTCTCCATGA GGAGCGCTGG   1020
```

```
CAGAATGGCC AATATAAACC AGGTATTGCC CACCGAGATC TGAGCAGCCA GAATGTGCTC   1080
ATTCGGGAAG ATGGATCGTG TGCCATTGGA GACCTGGGCC TTGCCTTGGT GCTCCCTGGC   1140
CTCACTCAGC CCCCTGCCTG GACCCCTACT CAACCACAAG GCCCAGCTGC CATCATGGAA   1200
GCTGGCACCC AGAGGTACAT GGCACCAGAG CTCTTGGACA AGACTCTGGA CCTACAGGAT   1260
TGGGGCATGG CCCTCCGACG AGCTGATATT TACTCTTTGG CTCTGCTCCT GTGGGAGATA   1320
CTGAGCCGCT GCCCAGATTT GAGGCCTGAC AGCAGTCCAC CACCCTTCCA ACTGGCCTAT   1380
GAGGCAGAAC TGGGCAATAC CCCTACCTCT GATGAGCTAT GGGCCTTGGC AGTGCAGGAG   1440
AGGAGGCGTC CCTACATCCC ATCCACCTGG CGCTGCTTTG CCACAGACCC TGATGGGCTG   1500
AGGGAGCTCC TAGAAGACTG TTGGGATGCA GACCCAGAAG CACGGCTGAC AGCTGAGTGT   1560
GTACAGCAGC GCCTGGCTGC CTTGGCCCAT CCTCAAGAGA GCCACCCCTT TCCAGAGAGC   1620
TGTCCACGTG GCTGCCCACC TCTCTGCCCA GAAGACTGTA CTTCAATTCC TGCCCCTACC   1680
ATCCTCCCCT GTAGGCCTCA GCGGAGTGCC TGCCACTTCA GCGTTCAGCA AGGCCCTTGT   1740
TCCAGGAATC CTCAGCCTGC CTGTACCCTT TCTCCTGTGT AAATATGCAG TTTATGTGTC   1800
ATCAATGTAC ATGCCAACAT AAATATGGCG ATT                                1833
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 573 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Domain
(B) LOCATION: 18..144
(D) OTHER INFORMATION: /note= "extracellular domain"

(ix) FEATURE:
(A) NAME/KEY: Domain
(B) LOCATION: 145..170
(D) OTHER INFORMATION: /note= "transmembrane region"

(ix) FEATURE:
(A) NAME/KEY: Domain
(B) LOCATION: 171..573
(D) OTHER INFORMATION: /note= "cytoplasmic domain"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Met Leu Gly Ser Leu Gly Leu Trp Ala Leu Leu Pro Thr Ala Val Glu
1 5 10 15

Ala Pro Pro Asn Arg Arg Thr Cys Val Phe Phe Glu Ala Pro Gly Val
20 25 30

Arg Gly Ser Thr Lys Thr Leu Gly Glu Leu Leu Asp Thr Gly Thr Glu
35 40 45

Leu Pro Arg Ala Ile Arg Cys Leu Tyr Ser Arg Cys Cys Phe Gly Ile
50 55 60

Trp Asn Leu Thr Gln Asp Arg Ala Gln Val Glu Met Gln Gly Cys Arg
65 70 75 80

Asp Ser Asp Glu Pro Gly Cys Glu Ser Leu His Cys Asp Pro Ser Pro
85 90 95

Arg Ala His Pro Ser Pro Gly Ser Thr Leu Phe Thr Cys Ser Cys Gly
100 105 110

Thr Asp Phe Cys Asn Ala Asn Tyr Ser His Leu Pro Pro Pro Gly Ser
115 120 125

Pro Gly Thr Pro Gly Ser Gln Gly Pro Gln Ala Ala Pro Gly Glu Ser
130 135 140

Ile Trp Met Ala Leu Val Leu Leu Gly Leu Phe Leu Leu Leu Leu Leu
145 150 155 160

Leu Leu Gly Ser Ile Ile Leu Ala Leu Leu Gln Arg Lys Asn Tyr Arg
165 170 175

Val Arg Gly Glu Pro Val Pro Glu Pro Arg Pro Asp Ser Gly Arg Asp
180 185 190

Trp Ser Val Glu Leu Gln Glu Leu Pro Glu Leu Cys Phe Ser Gln Val
195 200 205

Ile Arg Glu Gly Gly His Ala Val Val Trp Ala Gly Gln Leu Gln Gly
210 215 220

Lys Leu Val Ala Ile Lys Ala Phe Pro Pro Arg Ser Val Ala Gln Phe
225 230 235 240

Gln Ala Glu Arg Ala Leu Tyr Glu Leu Pro Gly Leu Gln His Asp His
245 250 255

Ile Val Arg Phe Ile Thr Ala Ser Arg Gly Gly Pro Gly Arg Leu Leu
260 265 270

Ser Gly Pro Leu Leu Val Leu Glu Leu His Pro Lys Gly Ser Leu Cys
275 280 285

His Tyr Leu Thr Gln Tyr Thr Ser Asp Trp Gly Ser Ser Leu Arg Met
290 295 300

Ala Leu Ser Leu Ala Gln Gly Leu Ala Phe Leu His Glu Glu Arg Trp
305 310 315 320

Gln Asn Gly Gln Tyr Lys Pro Gly Ile Ala His Arg Asp Leu Ser Ser
325 330 335

Gln Asn Val Leu Ile Arg Glu Asp Gly Ser Cys Ala Ile Gly Asp Leu
340 345 350

Gly Leu Ala Leu Val Leu Pro Gly Leu Thr Gln Pro Pro Ala Trp Thr
355 360 365

Pro Thr Gln Pro Gln Gly Pro Ala Ala Ile Met Glu Ala Gly Thr Gln
370 375 380

Arg Tyr Met Ala Pro Glu Leu Leu Asp Lys Thr Leu Asp Leu Gln Asp
385 390 395 400

Trp Gly Met Ala Leu Arg Arg Ala Asp Ile Tyr Ser Leu Ala Leu Leu
405 410 415

Leu Trp Glu Ile Leu Ser Arg Cys Pro Asp Leu Arg Pro Asp Ser Ser
420 425 430

Pro Pro Pro Phe Gln Leu Ala Tyr Glu Ala Glu Leu Gly Asn Thr Pro
435 440 445

Thr Ser Asp Glu Leu Trp Ala Leu Ala Val Gln Glu Arg Arg Arg Pro
450 455 460

Tyr Ile Pro Ser Thr Trp Arg Cys Phe Ala Thr Asp Pro Asp Gly Leu
465 470 475 480

Arg Glu Leu Leu Glu Asp Cys Trp Asp Ala Asp Pro Glu Ala Arg Leu
485 490 495

Thr Ala Glu Cys Val Gln Gln Arg Leu Ala Ala Leu Ala His Pro Gln
500 505 510

Glu Ser His Pro Phe Pro Glu Ser Cys Pro Arg Gly Cys Pro Pro Leu
515 520 525

Cys Pro Glu Asp Cys Thr Ser Ile Pro Ala Pro Thr Ile Leu Pro Cys
530 535 540

Arg Pro Gln Arg Ser Ala Cys His Phe Ser Val Gln Gln Gly Pro Cys
545 550 555 560

Ser Arg Asn Pro Gln Pro Ala Cys Thr Leu Ser Pro Val
565 570

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 900 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 1..109
(D) OTHER INFORMATION: /note= "exon 1"

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 110..413
(D) OTHER INFORMATION: /note= "intron A"

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 414..596
(D) OTHER INFORMATION: /note= "exon 2"

(ix) FEATURE:
(A) NAME/KEY: intron
(B) LOCATION: 597..834
(D) OTHER INFORMATION: /note= "intron B"

(ix) FEATURE:
(A) NAME/KEY: exon
(B) LOCATION: 835..900
(D) OTHER INFORMATION: /note= "exon 3 (partial)"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 597
(D) OTHER INFORMATION: /note= "nucleotide changed to A in patient T.A."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CCAGGGGCAG CTGTGCTGGC TTATGCTCTT CTCCTTCTGC TGCTGCCATC CTCCAGCAAG 60

ATGCTAGGGT CTTTGGGGCT TTGGGCATTA CTTCCCACAG CTGTGGAAGG TAAGTGTCTA 120

CAGGGAGGGG AAGGGTCTCT CCATCCATCC AGCAAGGGAA AGGGGCGCTT GAAGCAAGAG 180

CCACCCCTTT GGAAGAGTGG TGAGTGGGCT GGGTGAGTAA GGGTGAAGGA TAGAGCCATG 240

TGTCCCCATG GCAGGGCTCA GGTTCCAGGC CTCTGCTGAC CCTGCTTCCT CCTGTGGCTT 300

TACCATACTG ACGCTGGGAT GTGGAACATG TTTTGTCTAT TCTTTTGGCC AGTTTTTTGC 360

CTCTGCATTC ACTCCCACCT TGAATCTTTT CCTTTCCCCA CCCTGGGCCT CAGCACCCCC 420

AAACAGGCGA ACCTGTGTGT TCTTTGAGGC CCCTGGAGTG CGGGGAAGCA CAAAGACACT 480

GGGAGAGCTG CTAGATACAG GCACAGAGCT CCCCAGAGCT ATCCGCTGCC TCTACAGCCG 540

CTGCTGCTTT GGGATCTGGA ACCTGACCCA AGACCGGGCA CAGGTGGAAA TGCAAGGTGA 600

ATGGCAAAGT ATATGGCAGG TGATGGCTAG GGTGGGAGAC AGACACATCC TGGGGTGTGG 660

GTGGCAACCA AGGGGGAAGG GGAGAAATAG AACATCTGGT GGGAAAGAAA AGCCCATGAG 720

AGCTGGAAGG GACGCCTCTG ATAGAGAAGG GATTTACCCT CTGTTTCCAC ACCCCATTGT 780

GCTTTCTTCC TTGCCCCCCC TTTCTCTCCT CTTCCCCTAA TCCCATCCCA TCAGGATGCC 840

GAGACAGTGA TGAGCCAGGC TGTGAGTCCC TCCACTGTGA CCCAAGTCCC CGAGCCCACC 900

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..20
(D) OTHER INFORMATION: /note= "5' sense PCR primer 1s from human AMH receptor cDNA (nucleotides 36-55 of SEQ ID NO: 12)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TCTGCTGCTG CCATCCTCCA 20

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..21
(D) OTHER INFORMATION: /note= "3' antisense PCR primer 2a from human AMH receptor cDNA (complement of nucleotides 410-430 of SEQ ID NO: 12)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GCAGATGGCT GTAATTGGCA T 21

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..20
(D) OTHER INFORMATION: /note= "3' antisense PCR primer 3a from human AMH receptor cDNA (complement of nucleotides 504-523 of SEQ ID NO: 12)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ACAGCCCCAG CAGCACCAGT 20

Applicant's or agent's file reference number B174 CIP PCT	International application No.

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 27, lines 16-19.

B. IDENTIFICATION OF DEPOSIT E. coli, MC1061/P3/pB210 — Further deposits are identified on an additional sheet [x]

Name of depositary institution

American Type Culture Collection

Address of depositary institution *(including postal code and country)*

12301 Parklawn Drive
Rockville, Maryland 20852
United States of America

Date of deposit	Accession Number
16 December 1993 (16.12.93)	69520

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [X]

In respect of the designation of the EPO, samples of the deposited microorganisms will be made available until the publication of the mention of the grant of the European patent or until the date on which the application is refused or withdrawn or is deemed to be withdrawn, as provided in Rule 28(3) of the Implementing Regulations under the EPC only by the issue of a sample to an expert nominated by the requester (Rule 28(4) EPC).

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

EPO

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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[✓] This sheet was received with the international application	[ ] This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

Form PCT/RO/134 (July 1992)

Agent's file
reference number: B174 CIP PCT

Form PCT/RO/134

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

B. IDENTIFICATION OF DEPOSIT

E. coli, MC1061/P3/pB210, Accession Number 69520

Continuation of Box C.

In respect of the designation of Finland, until the application has been laid open to public inspection by the Finnish Patent Office, or has been finally decided upon by the Finnish Patent Office without having been laid open to public inspection, samples of the deposited microorganisms will be made available only to an expert in the art.

Continuation of Box D.

Finland

Applicant's or agent's file reference number B174 CIP PCT	International application No.

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 27, lines 19-22.

B. IDENTIFICATION OF DEPOSIT *E. coli*, MC1061/P3/pH1 — Further deposits are identified on an additional sheet [X]

Name of depositary institution

American Type Culture Collection

Address of depositary institution (including postal code and country)

12301 Parklawn Drive
Rockville, Maryland 20852
United States of America

Date of deposit	Accession Number
16 December 1993 (16.12.93)	69521

C. ADDITIONAL INDICATIONS (leave blank if not applicable) — This information is continued on an additional sheet [X]

In respect of the designation of the EPO, samples of the deposited microorganisms will be made available until the publication of the mention of the grant of the European patent or until the date on which the application is refused or withdrawn or is deemed to be withdrawn, as provided in Rule 28(3) of the Implementing Regulations under the EPC only by the issue of a sample to an expert nominated by the requester (Rule 28(4) EPC).

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

EPO

E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., 'Accession Number of Deposit')

For receiving Office use only	For International Bureau use only
[✓] This sheet was received with the international application	[ ] This sheet was received by the International Bureau on:
Authorized officer [illegible] DIVISION	Authorized officer

Form PCT/RO/134 (July 1992)

Agent's file
reference number: B174 CIP PCT

Form PCT/RO/134

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

B. IDENTIFICATION OF DEPOSIT

E. coli, MC1061/P3/pH1, Accession Number 69521

Continuation of Box C.

In respect of the designation of Finland, until the application has been laid open to public inspection by the Finnish Patent Office, or has been finally decided upon by the Finnish Patent Office without having been laid open to public inspection, samples of the deposited microorganisms will be made available only to an expert in the art.

Continuation of Box D.

Finland

Applicant's or agent's file reference number B174 CIP PCT	International application No.

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 34, line s 23-26.

B. IDENTIFICATION OF DEPOSIT Plasmid hAMHR3-2 — Further deposits are identified on an additional sheet ☐

Name of depositary institution

American Type Culture Collection

Address of depositary institution *(including postal code and country)*

12301 Parklawn Drive
Rockville, Maryland 20852
United States of America

Date of deposit	Accession Number
13 December 1994 (13.12.94)	

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☒

In respect of the designation of the EPO, samples of the deposited microorganisms will be made available until the publication of the mention of the grant of the European patent or until the date on which the application is refused or withdrawn or is deemed to be withdrawn, as provided in Rule 28(3) of the Implementing Regulations under the EPC only by the issue of a sample to an expert nominated by the requester (Rule 28(4) EPC).

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

EPO

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

Accession Number of Deposit

For receiving Office use only	For International Bureau use only
☑ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

Agent's file
reference number: B174 CIP PCT

Form PCT/RO/134

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

B. IDENTIFICATION OF DEPOSIT

Plasmid hAMHR3-2, Accession Number _____

Continuation of Box C.

In respect of the designation of Finland, until the application has been laid open to public inspection by the Finnish Patent Office, or has been finally decided upon by the Finnish Patent Office without having been laid open to public inspection, samples of the deposited microorganisms will be made available only to an expert in the art.

Continuation of Box D.

Finland

We claim:

1. An isolated DNA sequence comprising a DNA sequence encoding the mature AMH receptor polypeptide selected from the group consisting of

(a) nucleotides 113 to 1585 of SEQ ID NO: 1;

(b) nucleotides 110 to 1765 of SEQ ID NO: 2;

(c) nucleotides 112 to 1779 of SEQ ID NO: 12;

(d) DNA sequences that hybridize to any of the DNA sequences of (a)-(c) and which encode a biologically or antigenically active AMH receptor polypeptide; and

(e) DNA sequences that are degenerate to any of the foregoing DNA sequences.

2. An isolated DNA sequence according to claim 1 encoding the extracellular domain of the AMH receptor polypeptide selected from the group consisting of

(a) nucleotides 113 to 310 of SEQ ID NO: 1;

(b) nucleotides 110 to 490 of SEQ ID NO: 2;

(c) nucleotides 112 to 492 of SEQ ID NO: 12;

(d) DNA sequences that hybridize to any of the DNA sequences of (a)-(c) and which encode a biologically or antigenically active AMH receptor polypeptide; and

(e) DNA sequences that are degenerate to any of the foregoing DNA sequences.

3. An AMH receptor polypeptide encoded by the isolated DNA of claim 1.

4. An AMH receptor polypeptide according to claim 3 comprising an amino acid sequence corresponding to the mature AMH receptor polypeptide selected from the group consisting of

(a) amino acid residues 18 to 508 of SEQ ID NO: 3;

(b) amino acid residues 18 to 569 of SEQ ID NO: 4; and

(c) amino acid residues 18 to 573 of SEQ ID NO: 13.

5. An AMH receptor polypeptide according to claim 3 comprising an amino acid sequence corresponding to the extracellular domain the mature AMH receptor polypeptide selected from the group consisting of

(a) amino acid residues 18 to 83 of SEQ ID NO: 3;

(b) amino acid residues 18 to 144 of SEQ ID NO: 4; and

(c) amino acid residues 18 to 144 of SEQ ID NO: 13.

6. An antibody or antibody homolog which specifically recognizes an AMH receptor polypeptide according to claim 3.

7. An antibody or antibody homolog according to claim 6 which recognizes the extracellular domain of the AMH receptor polypeptide.

8. An antibody or antibody homolog according to claim 6 which recognizes the AMH receptor in its native conformation on the surface of cells.

9. An antibody or antibody homolog according to claim 6 which is conjugated to a further therapeutic agent selected from the group consisting of a toxin and a radionuclide.

10. A method of detecting the presence of an AMH receptor in a biological sample comprising the steps of

(a) contacting an antibody according to claim 6 with a biological sample suspected of containing an AMH receptor; and

(b) detecting immune complex formation between said antibody and a component of said biological sample, wherein said immune complex formation is indicative of the presence of an AMH receptor in said biological sample.

11. A method for detecting AMH ligand in a biological sample comprising the steps of

(a) contacting an AMH receptor polypeptide according to claim 3 with a biological sample suspected of containing an AMH ligand; and

(b) detecting binding between said polypeptide and a component of said biological sample, wherein said binding is indicative of the presence of an AMH ligand in said biological sample.

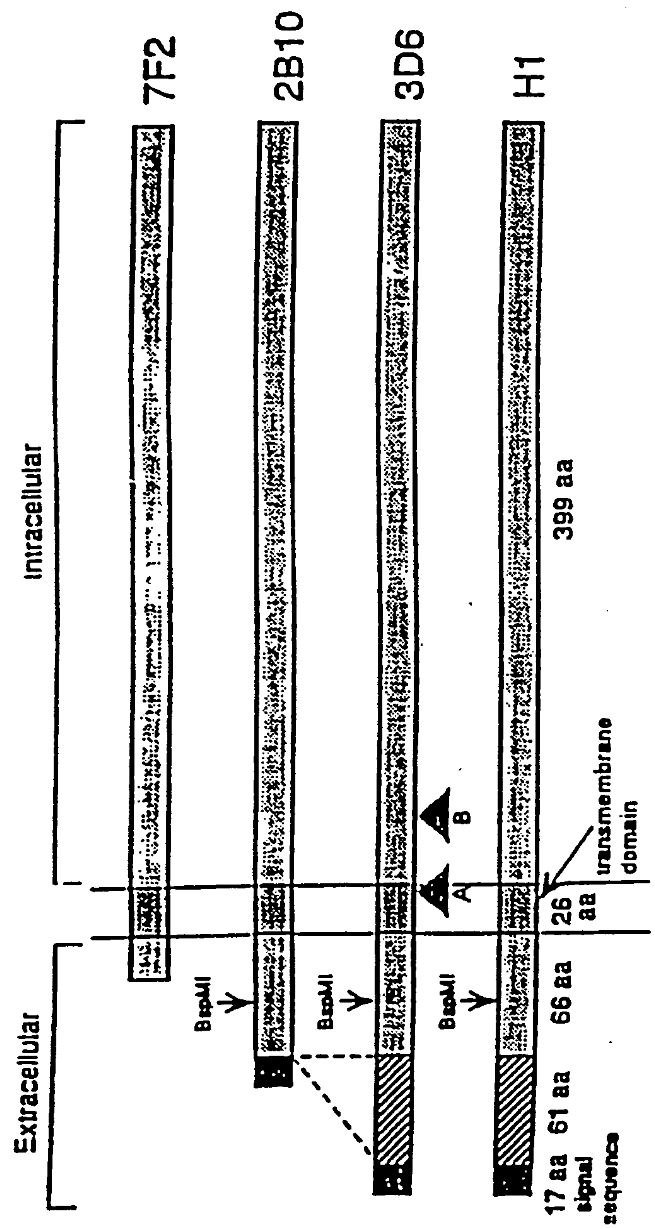

7F2
2B10
3D6
H1
Intracellular
Extracellular
BspMI
BspMI
BspMI
A
B
transmembrane domain
399 aa
26 aa
66 aa
61 aa
17 aa
signal sequence

FIG. 1A

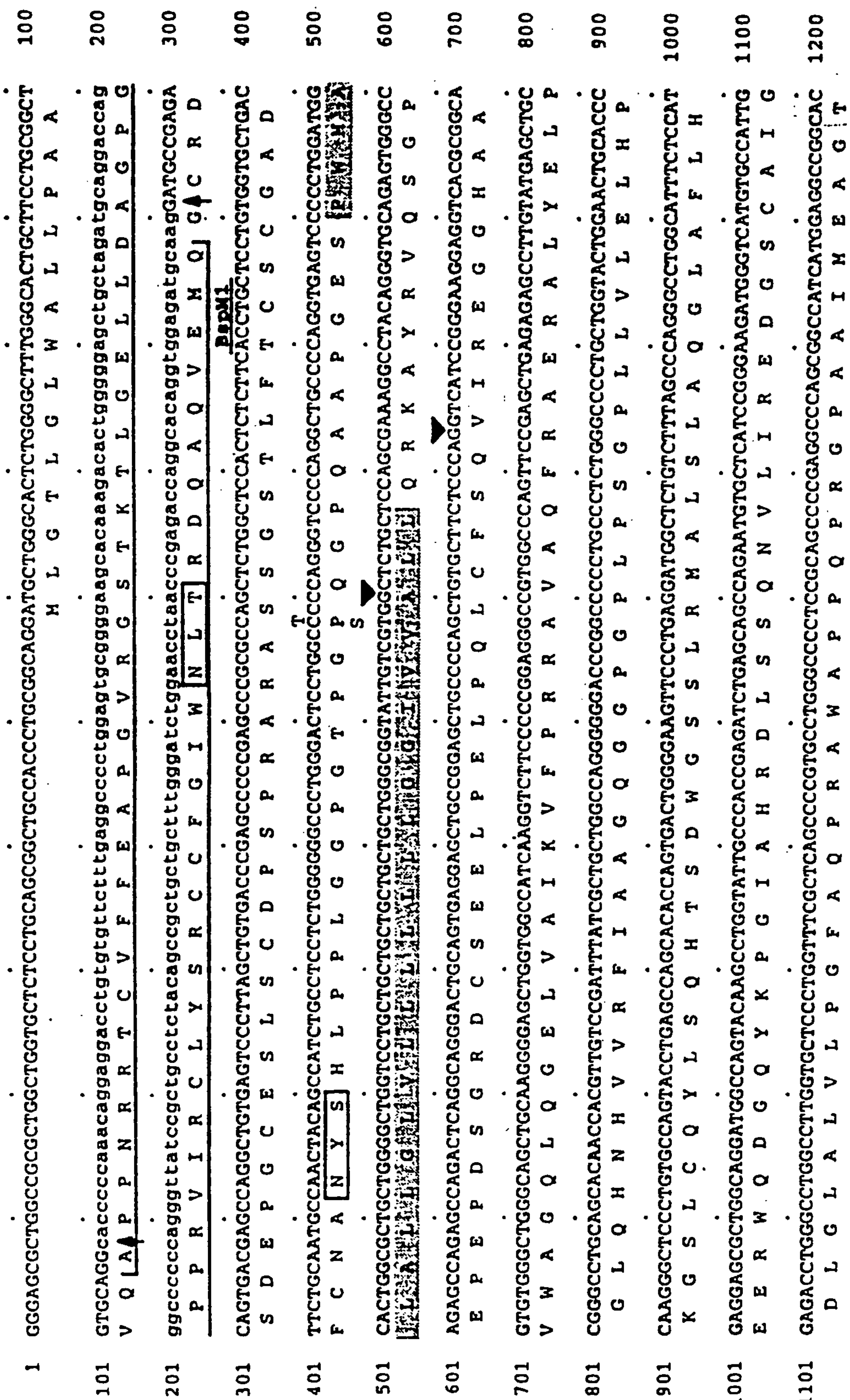


FIG. 1B

```
                 C
1201 ACAGAGGTACATGGCGCCAGAGCTCTTGGACAAGTCTCTGGACCTACAGGACTGGGGCACTGCCCTCCGGCGAGCCGACGTCTACTCCTTGGCCCTGCTC 1300
      Q  R  Y  M  A  P  E  L  L  D  K  S  L  D  L  Q  D  W  G  T  A  L  R  R  A  D  V  Y  S  U  A  L  L

1301 CTGTGGGAGATCCTGAGCCGCTGCCCGGATTTGAGGCCTGACGGCAGACCACCACCCTTCCAACTGGCCTATGAAGCAGAACTGGGCAGCGCCCCCACCA 1400
     L  W  E  I  L  S  R  C  P  D  L  R  P  D  G  R  P  P  P  F  Q  L  A  Y  E  A  E  L  G  S  A  P  T  T
                           S

1401 CCTGTGAGCTGTGGGCCCTGGCAGTAGAGGAGAGGAGGCGCCCTGACATCCCATCCTCCTGGTGCTGCTTTGCCACAGACCCCGGCGGGCTCAGGGAGCT 1500
       C  E  L  W  A  L  A  V  E  E  R  R  R  P  D  I  P  S  S  W  C  C  F  A  T  D  P  G  G  L  R  E  L

1501 GCTGGAAGACTGCTGGGACGCAGACCCGGAAGCGCGGCTGACGGCCGAGTGTGTCCAGCAGCGCCTGGTGGCCCTGGTTCATCCTCAGGAGGCCCAGCCC 1600
      L  E  D  C  W  D  A  D  P  E  A  R  L  T  A  E  C  V  Q  Q  R  L  V  A  L  V  H  P  Q  E  A  Q  P

1601 TGCCCAGAGGGCCGTCCACACAGCCACCCAGAAGACTGGCCCCCTGCTCCTGCCCCTGCCCCTGCTCTCCTCCCCGGCAGCCCACAGCCGGGTGCCTGCC 1700
     C  P  E  G  R  P  H  S  H  P  E  D  W  P  P  A  P  A  P  A  P  A  L  L  P  G  S  P  Q  P  G  A  C  H

1701 ACTTCGGTGTTCAGCAAGGCCTTTGCTCCAGGAACCCCGGAGCTGCCTGTGCCAGTTCTGACGTGTAAATAAGCAGTTTGTGTGTAATCTACCTGTAAAC 1800
       F  G  V  Q  Q  G  L  C  S  R  N  P  G  A  A  C  A  S  S  D  V

1801 GTAAACATGGCACTGGTATACCTGTCTGCTCTGCCTCTCCACTGTTTTCCCACTCCCCAATCTGTTAGGCAGGAAGCTGGAAATTGAGCCTATGTGTGTG 1900

1901 TGTGTGAGACAGGCCTGAAGACCAGCACCCATTGCAGAAGCCCCAGAAACCCAGCATCTTGCACTTCAGAGTCCTGCCCAGACCCTGCTAACCTCCCAGG 2000

                                                                                            End of 3D6
2001 TGGTCCCAGCCCTTCCCTCAAGGCAAACTCCCAGGAGAATTCTCTCTCCTCAGGACCAAAGGGGTTACCTGATCTGATA|ACACAGGGCATAAAACCTTCA 2100

2101 CAGAGTCCCCTTGCCAGCAAACCTGGGGAGGAATTTGCAAATTTTCACTCAACAAACCCTTCCACCAGGGCTCCCCCTTCTTTGTCCTGGAGGAGAAAGG 2200

2201 GAGGTGGGTAAACAGACTCCCTTAAAAACCTAGGGAGTCCAAACTGACCACGCACTCAGCCCTCTGCCTCTCTGCCTAGCCGCCCGCCTGGCCTGCCCAG 2300

2301 GTGTATTCTCTTCATTCAACCATGTGACCTTGCTCTTCCCCTGAGCGCTCTCTGTCTGTCCCCTCCGTTTTGACAGATGCCCTGTCCCCAATAAACCTTA 2400
                                                                                               Poly A Signal

2401 TCACTCTG   2408
```

FIG. 1C

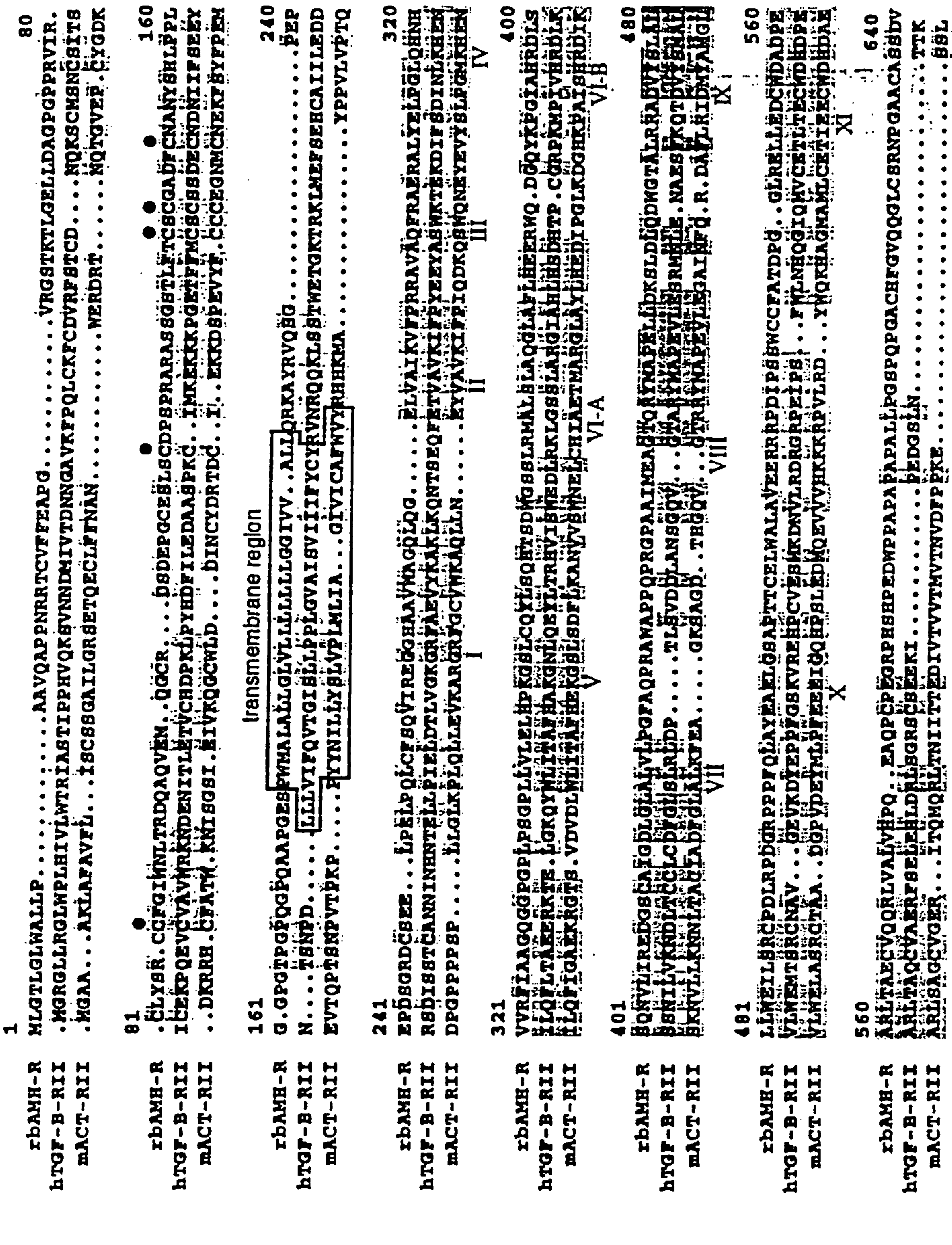


FIG. 1D

FIG. 2A

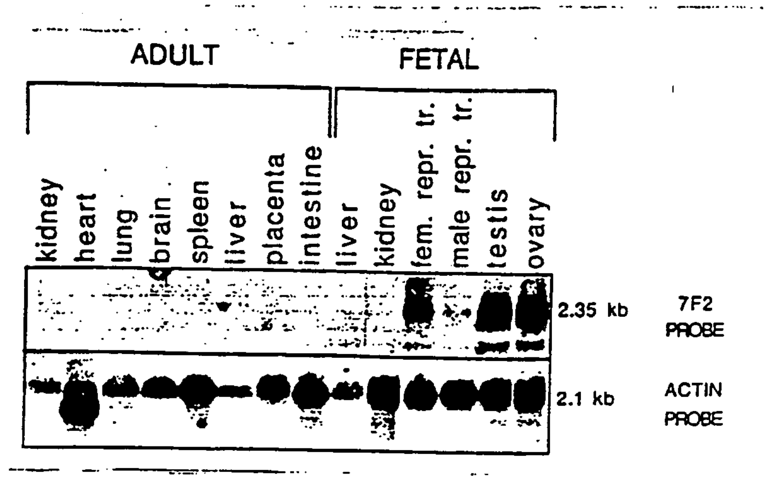

ADULT
FETAL
kidney
heart
lung
brain
spleen
liver
placenta
intestine
liver
kidney
fem. repr. tr.
male repr. tr.
testis
ovary
2.35 kb
7F2 PROBE
2.1 kb
ACTIN PROBE

FIG. 2B

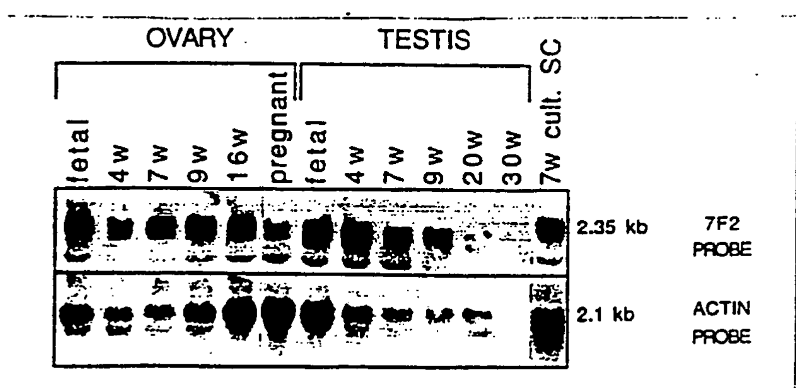
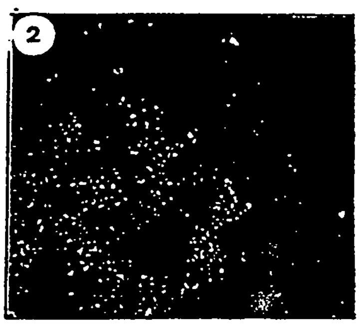

OVARY
TESTIS
fetal
4 w
7 w
9 w
16 w
pregnant
fetal
4 w
7 w
9 w
20 w
30 w
7w cult. SC
2.35 kb
7F2 PROBE
2.1 kb
ACTIN PROBE

FIG. 2C



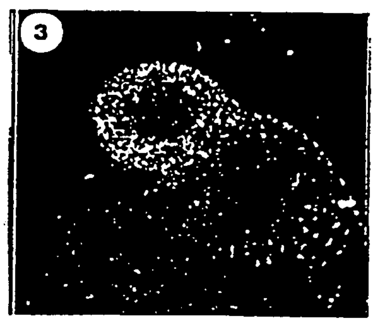


FIG. 2D FIG. 2E

FIG. 3A FIG. 3B

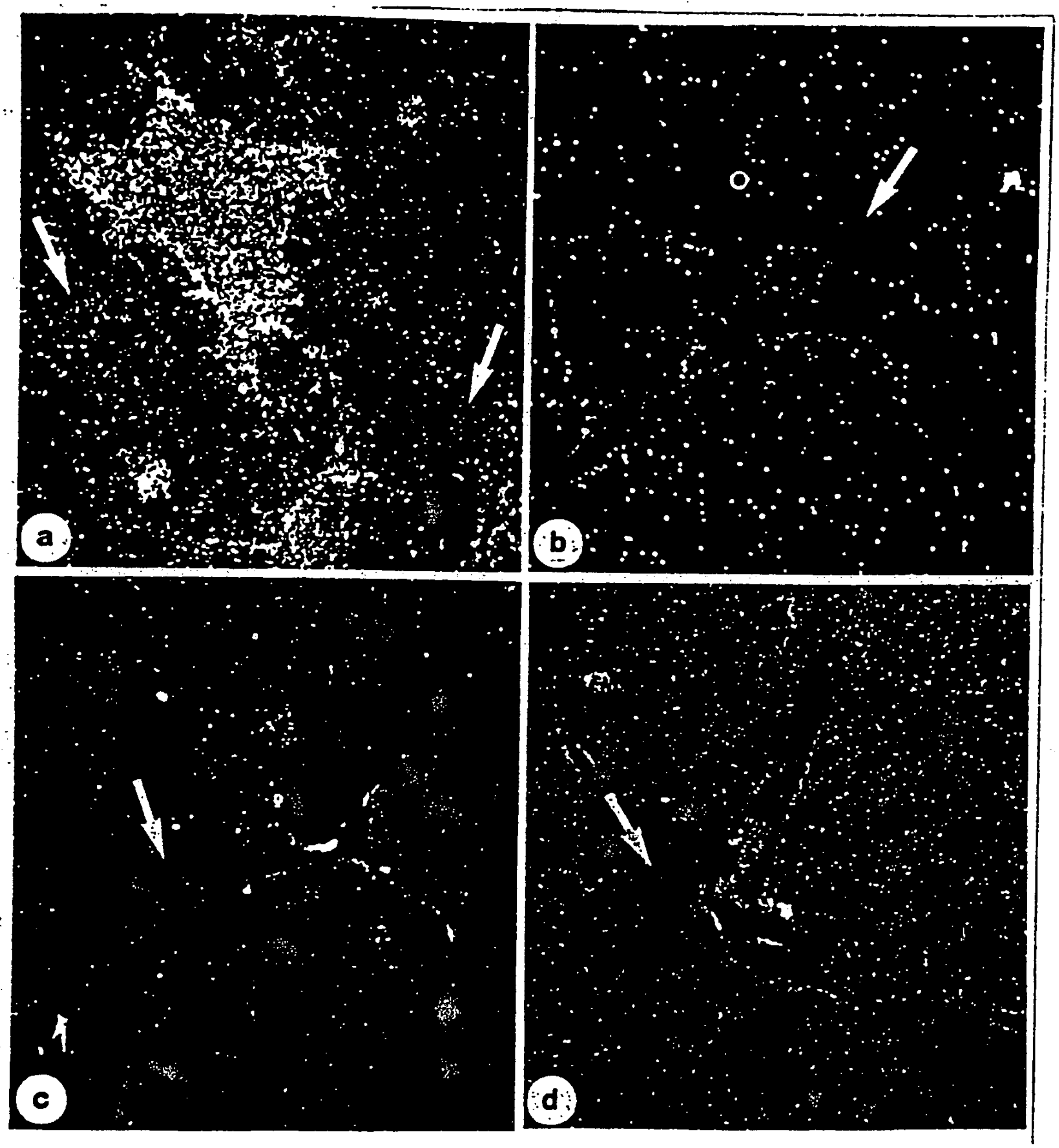


FIG. 3C FIG. 3D

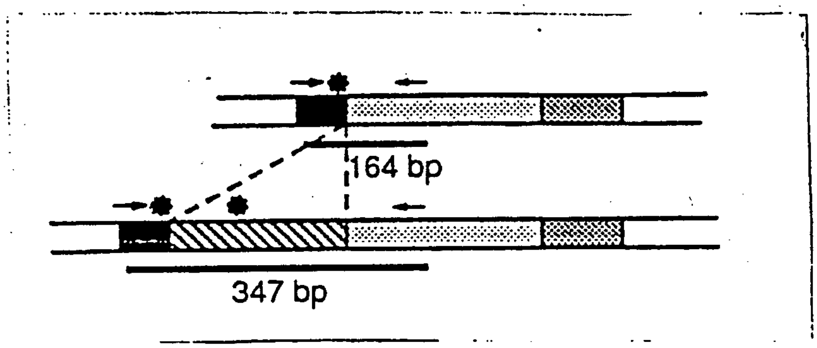

164 bp
347 bp

FIG. 4A

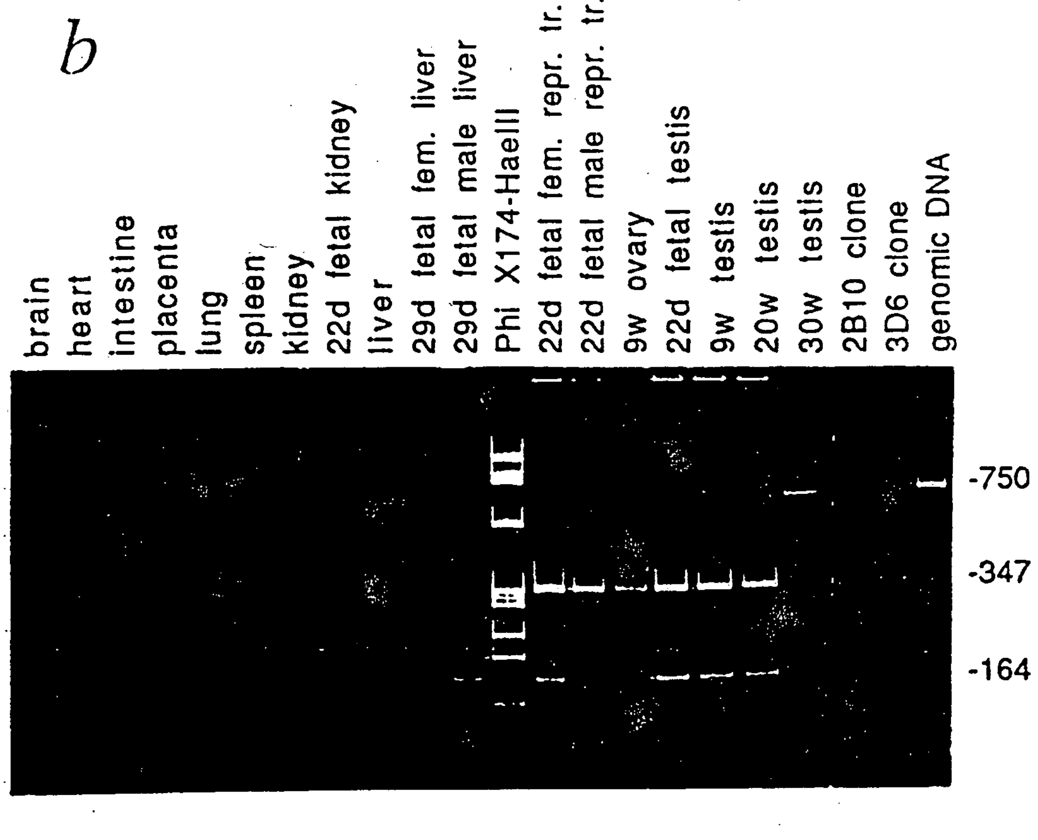

b
brain
heart
intestine
placenta
lung
spleen
kidney
22d fetal kidney
liver
29d fetal fem. liver
29d fetal male liver
Phi X174-HaeIII
22d fetal fem. repr. tr.
22d fetal male repr. tr.
9w ovary
22d fetal testis
9w testis
20w testis
30w testis
2B10 clone
3D6 clone
genomic DNA
-750
-347
-164

FIG. 4B

```
  1 MLGSLGLWALLPTAVEAPPNRRTCVFFEAPGVRGSTKTLGELLDTGTELP  50
    |||.|||||||||.||:|||||||||||||||||||||||||||||.|.: |
  1 MLGTLGLWALLPAAVQAPPNRRTCVFFEAPGVRGSTKTLGELLDAGPGPP  50

 51 RAIRCLYSRCCFGIWNLTQDRAQVEMQGCRDSDEPGCESLHCDPSPRAHP 100
    |.|||||||||||||||||.|.|||||||||||||||||| |||||||::
 51 RVIRCLYSRCCFGIWNLTRDQAQVEMQGCRDSDEPGCESLSCDPSPRARA 100

101 SPGSTLFTCSCGTDFCNANYSHLPPPGSPGTPGSQGPQAAPGESIWMALV 150
    |.||||||||||.|||||||||||| |:|||||.||||||||||| ||||.
101 SSGSTLFTCSCGADFCNANYSHLPPLGGPGTPGPQGPQAAPGESPWMALA 150

151 LLGLFLLLLLLLGSIILALLQRKNYRVRGEPVPEPRPDSGRDWSVELQEL 200
    ||||.||||||||:|::||||||.|||.::  ||| |||||| | ||.||
151 LLGLVLLLLLLLGGIVVALLQRKAYRVQSG..PEPEPDSGRDCSEELPEL 198

201 PELCFSQVIREGGHAVVWAGQLQGKLVAIKAFPPRSVAQFQAERALYELP 250
    |:||||||||||||.||||||||.|||||.||.|.||||.||||||||||
199 PQLCFSQVIREGGHAAVWAGQLQGELVAIKVFPRRAVAQFRAERALYELP 248

251 GLQHDHIVRFITASRGGPGRLLSGPLLVLELHPKGSLCHYLTQYTSDWGS 300
    ||||:|:||||.|:.||||.| |||||||||||||||:||.|.||||||
249 GLQHNHVVRFIAAGQGGPGPLPSGPLLVLELHPKGSLCQYLSQHTSDWGS 298

301 SLRMALSLAQGLAFLHEERWQNGQYKPGIAHRDLSSQNVLIREDGSCAIG 350
    ||||||||||||||||||||:|||||||||||||||||||||||||||||
299 SLRMALSLAQGLAFLHEERWQDGQYKPGIAHRDLSSQNVLIREDGSCAIG 348

351 DLGLALVLPGLTQPPAWTPTQPQGPAAIMEAGTQRYMAPELLDKTLDLQD 400
    ||||||||||:.||.||.|.||.|||||||||||||||||||||.|||||
349 DLGLALVLPGFAQPRAWAPPQPRGPAAIMEAGTQRYMAPELLDKSLDLQD 398

401 WGMALRRADIYSLALLLWEILSRCPDLRPDSSPPPFQLAYEAELGNTPTS 450
    || ||||||:||||||||||||||||||||:.||||||||||||||..||.
399 WGTALRRADVYSLALLLWEILSRCPDLRPDGRPPPFQLAYEAELGSAPTT 448

451 DELWALAVQERRRPYIPSTWRCFATDPDGLRELLEDCWDADPEARLTAEC 500
     ||||||:||||| |||.| ||||||:||||||||||||||||||||||||
449 CELWALAVEERRRPDIPSSWCCFATDPGGLRELLEDCWDADPEARLTAEC 498

501 VQQRLAALAHPQESHPFPESCPRGCPPLCPEDCTSIPAPTILPCRPQRSA 550
    |||||.||.||||.:| ||: |:: |.  |   ... |||.:||..||.:|
499 VQQRLVALVHPQEAQPCPEGRPHSHPEDWP..PAPAPAPALLPGSPQPGA 546

551 CHFSVQQGPCSRNPQPACTLSPV 573
    |||:|||| |||||.:||. |.|
547 CHFGVQQGLCSRNPGAACASSDV 569
```

FIG. 5

```
ccctgggcctcagCACCCCCAAACAGGCGAACCTGTGTGTTCTTTGAGGCCCCTGGAGTGCGGGGAAGCACAAAGACACTGGGAGAGCTGCTAGATACAG
             A  P  P  N  R  R  T  C  V  F  F  E  A  P  G  V  R  G  S  T  K  T  L  G  E  L  L  D  T  G

GCACAGAGCTCCCCAGAGCTATCCGCTGCCTCTACAGCCGCTGCTGCTTTGGGATCTGGAACCTGACCCAAGACCGGGCACAGGTGGAAATGCAAGatga
 T  E  L  P  R  A  I  R  C  L  Y  S  R  C  C  F  G  I  W  N  L  T  Q  D  R  A  Q  V  E  H  Q

atggcaaagtatatggcaggtgatggctagggtgggagacagacacatcctggggtgtgggtggcaaccaagggggaaggggagaaatagaacatctggt

gggaaagaaaagcccatgagagctggaagggacgcctctgatagagaagggatttaccctctgtttccacaccccattgtgctttcttccttgcccccc
```

FIG. 6

```
  1 CCAGGGGCAGCTGTGCTGGCTTATGCTCTTCTCCTTCTGCTGCTGCCATCCTCCAGCAAGATGCTAGGGTCTTTGGGGCTTTGGGCATTACTTCCCACAG 100
                                                                  M  L  G  S  L  G  L  W  A  L  L  P  T  A

101 CTGTGGAAGgtaagtgtctacagggaggggaagggtctctccatccatccagcaagggaaaggggcgcttgaagcaagagccacccctttggaagagtgg 200
     V  E

201 tgagtgggctgggtgagtaagggtgaaggatagagccatgtgtccccatggcagggctcaggttccaggcctctgctgaccctgcttcctcctgtggctt 300

301 taccatactgacgctgggatgtggaacatgttttgtctattcttttggccagtttttttgcctctgcattcactcccaccttgaatcttttcctttcccca 400

401 ccctgggcctcagCACCCCCAAACAGGCGAACCTGTGTGTTCTTTGAGGCCCCTGGAGTGCGGGGAAGCACAAAGACACTGGGAGAGCTGCTAGATACAG 500
              A  P  P  N  R  R  T  C  V  F  F  E  A  P  G  V  R  G  S  T  K  T  L  G  E  L  L  D  T  G

501 GCACAGAGCTCCCCAGAGCTATCCGCTGCCTCTACAGCCGCTGCTGCTTTGGGATCTGGAACCTGACCCAAGACCGGGCACAGGTGGAAATGCAAGgtga 600
      T  E  L  P  R  A  I  R  C  L  Y  S  R  C  C  F  G  I  W  N  L  T  Q  D  R  A  Q  V  E  M  Q  ↑

601 atggcaaagtatatggcaggtgatggctagggtgggagacagacacatcctggggtgtgggtggcaacccaagggggaaggggagaaatagaacatctggt 700

701 gggaaagaaaagcccatgagagctggaagggacgcctctgatagagaagggatttaccctctgtttccacaccccattgtgctttcttccttgccccccc 800

801 tttctctcctcttcccctaatcccatcccatcagGATGCCGAGACAGTGATGAGCCAGGCTGTGAGTCCCTCCACTGTGACCCAAGTCCCCGAGCCCACC 900
                                     G  C  R  D  S  D  E  P  G  C  E  S  L  H  C  D  P  S  P  R  A  H
```

FIG. 7

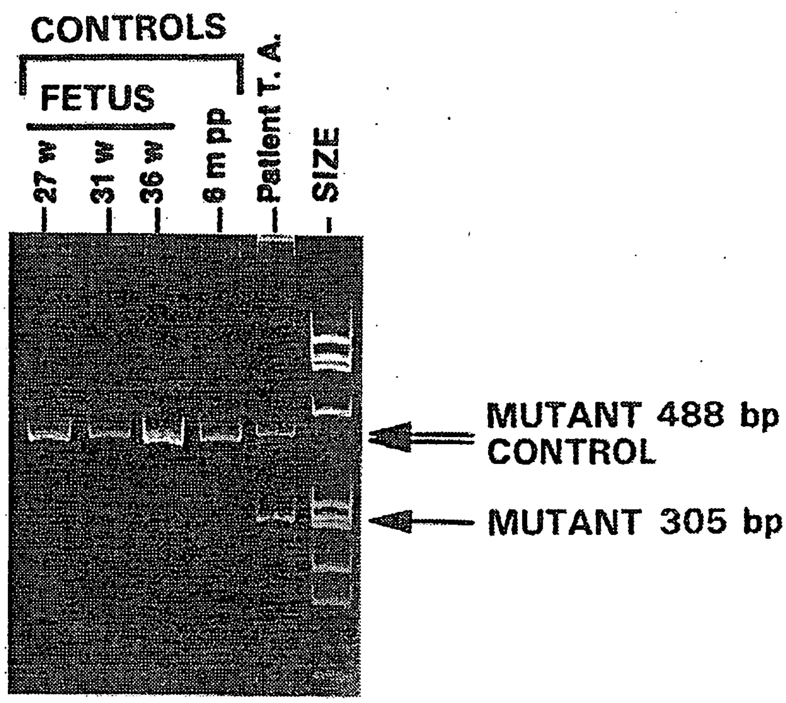
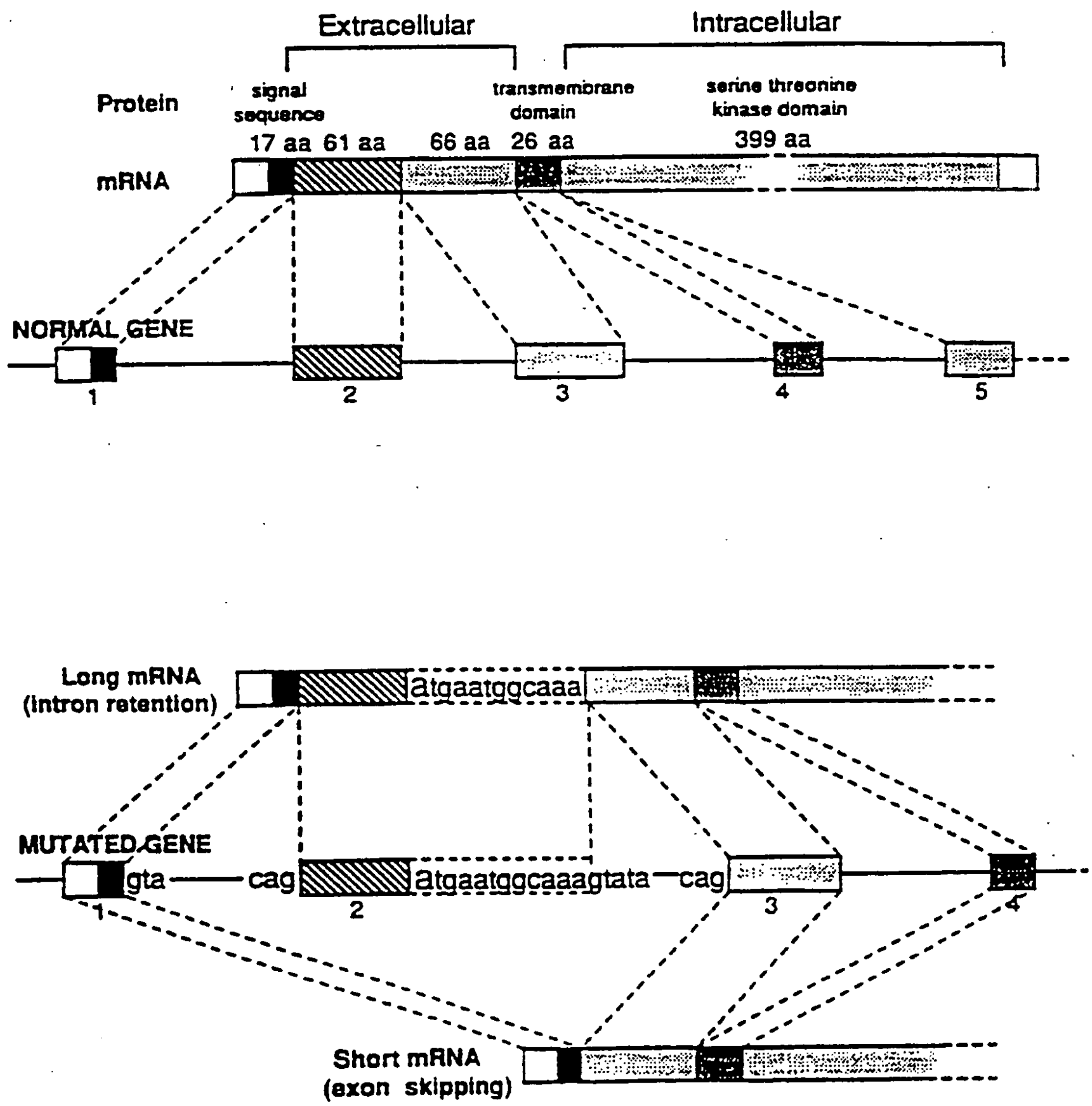

CONTROLS
FETUS
27 w
31 w
36 w
6 m pp
Patient T. A.
SIZE
MUTANT 488 bp
CONTROL
MUTANT 305 bp

FIG. 8

FIG. 9


Extracellular
Intracellular
Protein
signal sequence
transmembrane domain
serine threonine kinase domain
17 aa
61 aa
66 aa
26 aa
399 aa
mRNA
NORMAL GENE
1
2
3
4
5
Long mRNA (intron retention)
atgaatggcaaa
MUTATED GENE
gta
cag
atgaatggcaaagtata
cag
1
2
3
4
Short mRNA (exon skipping)

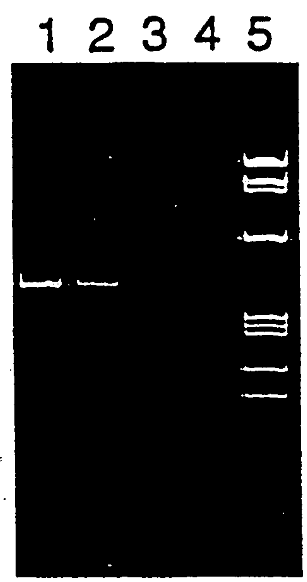


FIG. 10

Applicant's or agent's file reference number B174 CIP PCT	International application No.

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 34, lines 23-26

B. IDENTIFICATION OF DEPOSIT Plasmid hAMHR3-2 — Further deposits are identified on an additional sheet ☐

Name of depositary institution

American Type Culture Collection

Address of depositary institution *(including postal code and country)*

12301 Parklawn Drive
Rockville, Maryland 20852
United States of America

Date of deposit	Accession Number
13 December 1994 (13.12.94)	69719

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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A3

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08/173,512	23 December 1993 (23.12.93)	US

(60) Parent Applications or Grants

(63) Related by Continuation

US	08/166,333 (CIP)
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Filed on	23 December 1993 (23.12.93)

(71) Applicants (*for all designated States except US*): BIOGEN, INC. [US/US]; 14 Cambridge Center, Cambridge, MA 02142 (US). INSERM (U.293) [FR/FR]; 1, rue Maurice-Arnoux, F-29120 Montrouge (FR).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): CATE, Richard, L. [US/US]; 14 Cambridge Center, Cambridge, MA 02142 (US). JOSSO, Nathalie [FR/FR]; INSERM (U.293), 1, rue Maurice-Arnoux, F-29120 Montrouge (FR).

(74) Agents: HALEY, James, F., Jr. et al.; Fish & Neave, 1251 Avenue of the Americas, New York, NY 10020-1104 (US).

(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

With an indication in relation to a deposited microorganism furnished under Rule 13bis separately from the description.

Date of receipt by the International Bureau:

27 January 1995 (27.01.95)

(88) Date of publication of the international search report: 10 August 1995 (10.08.95)

(54) Title: ANTI-MULLERIAN HORMONE RECEPTOR POLYPEPTIDES AND ANTIBODIES THERETO

(57) Abstract

This invention relates to polypeptides displaying the activity of anti-Mullerian hormone (AMH) receptor, also known as Mullerian inhibiting substance (MIS) receptors, and antibodies to those polypeptides. More particularly, this invention relates to such AMH receptor polypeptides and antibodies, processes for producing those polypeptides and antibodies and methods for using them in the treatment of cancer and tumors of tissues associated with expression of the anti-Mullerian hormone receptor.

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GA	Gabon				

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US/94/14643

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/71 C07K14/72 C07K16/28 G01N33/74

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GenBank database entry, submitted 27-04-93 Accession number X71916 BAARENDS, W.M. et al.: 'A novel member of the transmembrane serine/threonine kinase receptor family ...' , see the abstract & DEVELOPMENT, vol. 120,no. 1, 1994 pages 189-197, ---	1-11
A	WO,A,93 19177 (THE GENERAL HOSPITAL CORP.) 30 September 1993 * claims; p. 1-10 * ---	1-11

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1

Date of the actual completion of the international search	Date of mailing of the international search report
9 June 1995	20.06.95
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Authorized officer Hermann, R

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Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHEMICAL ABSTRACTS, vol. 120, no. 11, 14 March 1994 Columbus, Ohio, US; abstract no. 128396, HE, W.W. ET AL. 'Developmental expression of four novel serine/threonine kinase receptors homologous to the activin/transforming growth factor beta type II receptor family' see abstract & DEV.DYN., vol. 196,no. 2, 1993 pages 133-142, ---	1-11
A	CHEMICAL ABSTRACTS, vol. 120, no. 5, 31 January 1994 Columbus, Ohio, US; abstract no. 46292, CATLIN, E. ET AL. 'Identification of a receptor for human Mullerian inhibiting substance' see abstract & ENDOCRINOLOGY, vol. 133,no. 6, 1993 pages 3007-30013, ---	1-11
P,X	CHEMICAL ABSTRACTS, vol. 121, no. 1, 4 July 1994 Columbus, Ohio, US; abstract no. 1123, GROOTEGOED, J.A. ET AL. 'Welcome to the family: the anti-mullerian hormone receptor' see abstract & MOL. CELL. ENDOCRINOL., vol. 100,no. 1-2, 1994 pages 29-34, ---	1-11
P,X	CHEMICAL ABSTRACTS, vol. 121, no. 19, 7 November 1994 Columbus, Ohio, US; abstract no. 222195, DI CLEMENTE, N. ET AL. 'Cloning, expression and alternative splicing of the receptor for anti-mullerian hormone' see abstract & MOL. ENDOCRINOL., vol. 8,no. 8, 1994 pages 1006-1020, -----	1-11

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter nal Application No
PCT/US 94/14643

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9319177	30-09-93	AU-B- 3920693	21-10-93